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THE INTERNATIONAL ACADEMY OF GYNECOLOGICAL CYTOLOGY

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ACADEMIA INTERNACIONAL DE CITOLOGIA GINECOLOGICA

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BUSINESS MATTERS OF THE INTERNATIONAL ACADEMY

FROM THE OFFICE OF THE PRESIDENT

In accordance with the Bylaws of the International Academy Article I, Section 3, an

INTERNATIONAL MEETING ON EXFOLIATIVE CYTOLOGY

is being planned for the year 1961. The place of the meeting probably will be Vienna, Austria.

Extracts from the Bylaws concerning the Scientific Session (*Article 1, Section 10*) : ". . . Papers presented at the Scientific Session shall be original papers which have never been presented or published. Material which has already been published or presented elsewhere may be considered in panel discussions. Fifty per cent of the Scientific Session shall be devoted to original papers, and fifty per cent of the time to panel discussions. . . ."

For details concerning the International Meeting write to Dr. Ruth M. Graham, Roswell Park Park Memorial Institute, 666 Elm Street, Buffalo 3, New York, U.S.A.

LETTERS TO THE EDITORS

REMARKS CONCERNING THE ACTION OF CERTAIN NON-VIRILIZING ANDROGENS ON THE VAGINAL EPITHELIUM

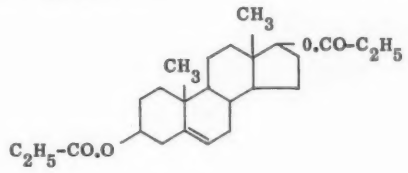
TO THE EDITORS:

The non-virilizing androgens are substances with an "anti-estrogenic" effect. Ferin (1) has already observed this action in the uteri of two ovariectomized women whose sensibility to estrogenic substances was well known. It seems, however, that this notion has been incorrectly applied to other receptors.

On the other hand, Ferin stated that vaginal smears also revealed an estrogenic response to the non-virilizing androgens which remained localized to the vaginal epithelium. Although this statement has been experimentally verified, the property which the non-virilizing androgens possess of exercising a proliferative action of maturity, as estrogens do, is usually not discussed. The vaginal cytology necessarily reflects this influence.

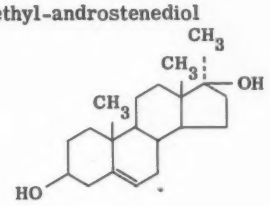
We have studied the action of various derivatives on the vaginal epithelium of the castrated and non-castrated rat.

Androstenediol 3, 17-dipropionate



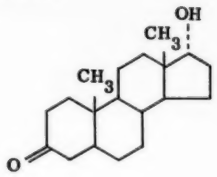
with the usual doses (300 mg. in 6 days) causes vaginal keratinization in every single case (Fig. 1).

On the contrary, 17-α-methyl-androstenediol



keratinizes only the vagina of the castrated animal; 300 mg. in 6 days does not prevent the cycle and vaginal modification from occurring.

Finally, androstane-3-one-17α-ol



studied by both Ferin (1) and Flamand, Simon and Spehl (3), has shown extraordinary estrogenizing effects in castrated as well as in intact animals (with 120 mg. in 6 days). Once more, however, we have noticed that the keratinization was much more pronounced in the rats without ovaries.

The use of these same hormones in treating patients had led us to similar conclusions. Following the administration of 100-150 mg. of one of these hormones, all signs of follicular maturity, accompanied by elevation of the Eosinophilic and Karyopyknotic Indices, appeared in almost every case of castrated or menopausal women with atrophic vaginal smears initially.

This estrogenizing action of non-virilizing androgens, well known as "anti-estrogens," should be recognized, as it has been proven by experiences on humans as well as animals.

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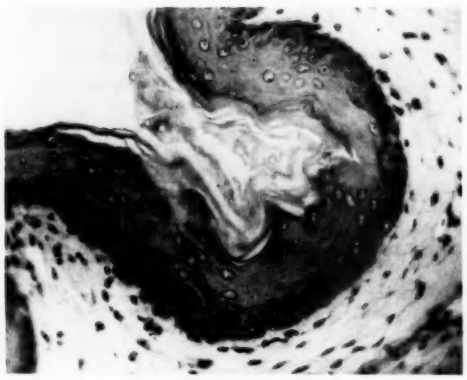

Fig. 1

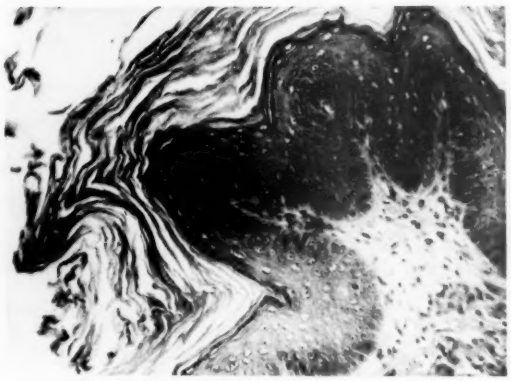

Fig. 2

CYTOLOGICAL TERMINOLOGY

TO THE EDITORS:

With all due respect to the distinguished group of scientists who undertook the tremendous and evidently time consuming task of establishing a uniform nomenclature pertaining to the exfoliative cytology of the female genital tract, this writer finds himself in disagreement with some of the terminology suggested and the reasoning behind it, and fails to see an advantage in the effort to modify the concepts established by Papanicolaou. The desire to pinpoint exactly the origin of a squamous cell and its "layer status" is a valid one, but unfortunately based on false premises. When Papanicolaou designated squamous cells as superficial, intermediate, parabasal, and basal, he referred to an intact, mature squamous epithelium of the vagina and cervix. In reality, all cells that desquamate spontaneously from the surface of the epithelium, or for that matter are removed by means of a cotton swab, are surface cells and therefore superficial. What in essence varies is not the layer of origin of these cells but their degree of maturity. The only correct designation of squamous cells in conformity with their physiologic status would therefore be:

1. Cells of the germinative layer.

2. Immature squamous cells.

3. Maturing or mature squamous cells with the degree of maturity specified.

In practice, however, the writer and many others found it convenient to use Papanicolaou's terminology which is an easy one and convenient to use and has a vivid descriptive value.

Therefore, the fact that an essentially incorrect terminology was discussed by the members of the Terminology Subcommittee, subtracts much of the value to the arguments concerning the exact designation of the cells and there is also little hope that following this effort, a completely uniform terminology will be achieved. It is also hoped that the Editor will reconsider his statement that only one terminology will be acceptable for publication in ACTA CYTOLOGICA. In order to achieve this, each designation of cells would have to be accompanied by a photograph and this would render all publication nearly impossible. May I plead the cause of the non-conformists and beg that a place be reserved for them in ACTA CYTOLOGICA ?

It would also be rather dangerous to get entangled into the language problems concerning the cells. Unless Latin is going to be used, there will always be problems of translation. Since English is the language of choice, I would be careful in accepting some of the suggested changes based on linguistic feelings of individual participants.

To come to the point, there seems to be a significant amount of confusion among the participants concerning the designation of "cornified" cells. I do not believe that Papanicolaou ever meant to imply that the cornified cell contained horn. For keratin or horn containing cells are mature superficial squamous cells with eosinophilic cytoplasm. The blue superficial cells are the "precornified" cells and I find both these terms most convenient. Indeed, no one suggested that the acidophilic cells turn blue, but obviously, the blue cells, if given a chance, turn pink. Thus I vote to retain this part of the Papanicolaou nomenclature.

Much of the discussion concerning the differentiation between the intermediate and superficial cells also belongs to the same category of dialectic argument. Physiologically, many of the superficial cells have vesicular nuclei and yet the cytoplasmic characteristics of mature superficial cells. Biopsies of the squamous mucosa obtained at the same time show perfectly mature squamous epithelium with piling up of many superficial layers. Should we assume that the obviously superficial cells are not superficial after all ? And if they are intermediate, what is above them ? I vote that the term "intermediate cells" be reserved for the smaller, less mature squamous cells of navicular appearance with opaque cytoplasm, according to Papanicolaou's definition.

I found the term "squame" grammatically incorrect. My dictionary carries the word "squama" of Latin origin and meaning a scale. The term "keratinized cell" still appears unsatisfactory. The term "metaplastic cells" does not conform with the realities of tissue pathology. In order for a cell to be metaplastic, it has to show evidence of transformation from one type of cell to another. This is obviously difficult if not impossible to demonstrate, and even in tissue pathology where a great deal more evidence is available, the problem has not been solved to the entire satisfaction of everybody concerned. Those among us who read Robert Meyer's writing with respect and piety would still prefer the non-committal term of "epidermoid differentiation" in reference to the endocervix. The cells under discussion are simply immature squamous epithelial cells and may have a multitude of origins. The same applies to the term "erosion cells." Erosion, or better, erythroplakia (Navratil), like leukoplakia, is purely a clinical designation, and in gynecologic reality, corresponds more frequently than not, to an eversion of the endocervical mucosa over the surface of the portio. The only cells coming from such a surface, are columnar endocervical cells. The immature squamous cells may also originate from the surface of a superficial ulceration of the epithelium which, admittedly may also present itself clinically as an erosion. The term "erosion cells" is misleading and too all-inclusive to be acceptable.

I believe that the effort was well worth-while and disclosed that, while some attempts at new designations were being made, much of the thinking among the distinguished cytologists was along the same lines, and the differences of opinion were relatively minor ones. The writer is obviously prejudiced and would like to see Papanicolaou's work maintained intact until some startling discoveries come our way.

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Reference is made to Dr. Koss' above remarks concerning the discussions on terminology.

Personally, I cannot follow Dr. Koss in his comments. Every cytologist, I am convinced, has a great admiration for the splendid work of Dr. Papanicolaou, but that is not an argument for blindly accepting his terminology without criticism, especially since he himself agrees to discuss and change the terminology. One should not forget that cytology as well as, for example, endocrinology, is a new science with an important, continuous evolution. So it is inevitable that further research or further practical experience will show from time to time that some theories or terminologies must be changed or brought into accord with the new findings. Cytology is not a science which can be studied by one single person or laboratory. It is impossible for a single person to do all the research necessary to clear up the problems. This is only possible when research is done by several workers, each interested in a more detailed part of cytology; by putting together these results after critically examining them, we can progressively construct the basis of cytology. But this harmonious construction is possible only if the different research papers can be read and understood by everybody. This implies the necessary understanding that every cytologist use the same terminology.

It is most important that we arrive at a precise terminology that will permit a general understanding. Unfortunately, the referendum shows that at the present time there does not yet exist a uniformity in terminology and interpretation, so that what one cytologist understands by a term may be contrary to the interpretation of another cytologist. This applies especially to the terms "cornification," "keratinization" and "superficial cells."

If the original terminology is so incorrect, as pointed out by Dr. Koss, I think that this is one of the chief arguments for changing it, and not an argument to stop discussions at the beginning. Let us follow a more logical policy. At first we must get a precise definition, and then, if everybody will accept this definition for a given term, we can change the original term to a more correct term.

For example, would it not be more logical and useful to obtain for the superficial cell at first a general understanding of the concept and the criteria for diagnosis of such a cell, so that every cytologist will understand by this term the same cell type and not another. Finally, if we have obtained such a uniformity in definition and diagnosis, the most important problem is resolved and we now have the time to discuss the accuracy of the term. I see no particular reason for not changing the term "superficial cell" if it is necessary for scientific, logical or practical reasons. However, before we change a term which is also used in general histology, we should see that the term is also changed in histology, not only in one field of science, and with international agreement.

Dr. Koss pleads the cause of the non-conformists and requests that a place be reserved for them in ACTA CYTOLOGICA. May I stress that such a policy could be a greater danger to ACTA CYTOLOGICA and cytology in general. What would be the evolution of endocrinology, for example, if everybody had asked and defended the same policy of non-conformity or asked that endocrinology should strictly retain the original terminology of the pioneers ? As long as every laboratory used its own terminology and units it was not possible to obtain serious results. The great evolution of endocrinology started at the moment international meetings fixed international terminology and international units so that everybody in the world understood by the same term the same thing. Hence controlled research work could be accomplished, while before this time there existed general confusion with unnecessary polemics.

Today, non-conformity, in medicine as well as in politics, is the serious problem which blocks the solution of most problems, so that an international understanding is not only necessary but of vital importance. Non-conformity is defendable only for exceptional persons or occasions, but not in problems of international importance, even if conformity is possible only by abandoning some personal prestige.

For these reasons, I still support the efforts of the Terminology Sub-Committee, and I strongly ask that the I.A.G.C. avoid the tendency toward non-conformity. Otherwise, the I.A.G.C. has no reason for existence, if everybody will not first accept the necessity of a general international

understanding which must predominate over personal prestige. The International Academy has the choice between a uniform terminology for ACTA CYTOLOGICA, so that everybody will be able to understand the papers, the only way to obtain any serious discussions, or a terminology of non-conformity, inviting everyone to use his own terminology. What we need in medicine as well as in international diplomatic relations is general understanding and not general confusion. The International Academy should be the place where this general understanding is the first rule, and not a second tower of Babel.

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DEFINITION OF DYSKARYOSIS

TO THE EDITORS:

Reference is made to the letter to the Editor by Dr. José del Sol in Volume II, Number 1, 1958, page 12-14.

As one who does not utilize the concept of "dyskaryosis" I would like to comment on the questions raised by Dr. del Sol, although replies from Drs. Graham, Papanicolaou and others who accept the term "dyskaryosis" may be regarded as more authoritative.

A variety of changes may exist in the mucosa of the uterine cervix at any given moment. Only by considering the overall cellular evidence can one hope to determine the nature of the underlying pathological process in the uterine cervix. The cellular changes which are included under the heading of "dyskaryosis" are observed in patients with a variety of histopathological changes in the surface mucosa and may even be observed in women with frank cancer.

In replying to question # 1, the cells depicted in figure # 1 are from a focus of atypical epithelium, while those of figure # 2 are more dedifferentiated, and their surface change of origin would be designated as carcinoma in situ by most pathologists. Although changes depicted in both figures might be observed in cell films from women with cancer, there is no evidence to suggest this in the photomicrographs presented.

In answering question # 2, it is important to remember that most pathological processes are dynamic. At one site changes may be more advanced while elsewhere they are less striking. This must be taken into consideration in evaluating cellular evidence. When several processes co-exist it is more important to recognize the more advanced change. As indicated the cells of figure # 1 do not suggest cancer, while the cells of figure # 2 are probably from a surface change which most pathologists would designate as carcinoma in situ.

The cell of figure # 3 referred to in the third question originated in an epithelium whose surface cells are capable of differentiation as manifested by the size of the cytosome. Similarly, these surface cells possess a relatively large nuclear mass which is quite abnormal for this level in the epithelium. The nuclear mass appears translucent, indicating a degenerative change. Such a change may co-exist with a cancer, but the depicted evidence does not suggest cancer.

Measurements of cells and nuclei from many different processes in the uterine cervix indicate that most of the cells which are included in the category of dyskaryosis fall within the range of the normal. A few are larger than the largest recorded normal cells. Some 5000 cells which others would include in the category of dyskaryosis had a mean area of 1519 + 611.34 square microns as compared with a normal value of 1604.15 square microns. Rare cells are larger and nuclear masses up to 700 square microns in area have been measured. The basic defect is essentially the same in the giant forms as in those falling within the normal range, and therefore the nuclear-cytoplasmic ratio is probably more important in evaluating the cells.

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CRYSTALS IN SMEARS

TO THE EDITORS:

I just received the issue of the ACTA CYTOLOGICA in which you publish a picture of crystals found at times in smears (Volume II, Number 1, page 24). We have seen these crystals not infrequently in smears taken on women who have used certain contraceptive jellies. They are often encountered in those cases in which the clinical harmlessness of contraceptives is being tested by putting a contraceptive into a cervical cap which is put onto the cervix for 24 hours. Smears prepared from the content of the cap (which contains cells desquamated in the last 24 hours as well as the contraceptive) show the crystals you photographed when certain contraceptives are used. The crystals were also found when the contraceptive alone was smeared as well as in spreads prepared from a starch paste. We therefore presume that a smear containing these crystals was taken from a woman who has used a certain brand of contraceptive jelly.

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THE SYMPOSIA BY CORRESPONDENCE OF ACTA CYTOLOGICA

INTRODUCTORY REMARKS

The Symposia of ACTA CYTOLOGICA are *held entirely by correspondence* and contain international discussions of scientific problems of interest to the exfoliative cytologist.

System for Selecting Subjects for Symposia: From recommendations received, the Editorial Office will draw up the list of subjects and will publish these subjects in ACTA CYTOLOGICA, under the heading FUTURE SYMPOSIA.

The final detailed program will be published in ACTA CYTOLOGICA immediately preceding the one where these topics are to be considered, under the heading, THE NEXT SYMPOSIUM.

Instructions for Authors: Each problem will be introduced by a *Main Speaker* or *Speakers.* These principal papers will then be considered by persons identified as *Discussants.* As a general rule, approximately 600 words each will be allocated for main papers and 200 words each will be allocated for the contributions of the Discussants. The Main Speakers will then be given the opportunity to make unlimited Closing Remarks.

Photomicrographs and tables may be reproduced: one full page for each principal paper and for the paper of the Discussant (maximum one-half page per contribution). The photomicrographs and tables should be submitted in glossy photographic prints, preferably in the size of 5 × 7 inches (i.e., 12 × 18 cm) and should show a proportional 10μ scale on its reverse side. *Each figure should be accompanied by a comprehensive caption.*

The Discussants are requested *to strictly restrict their contributions to the discussion of the main papers.* Discussions which are not directly related to the main paper cannot be accepted. It is suggested that the Discussants prepare their contributions in such a manner that the reader may gain the impression of an actual round table conference.

The Closing Remarks of the Main Speakers should be limited to the answering of questions raised in the discussion and to other directly related information.

The Bibliography for the papers of both Main Speaker and Discussant should be organized in the same manner as in the American Journal of Obstetrics and Gynecology, at the *end* of the paper. *Every cited opinion or publication should have a reference in the bibliography.*

Deadline for Contributions: The Editorial Office will set deadlines for each written symposium. These will include:

1. deadline for agreements to contribute.
2. deadline for main papers.
3. deadline for discussions.
4. deadline for closing remarks.

Reprints: Authors may receive reprints of their papers by ordering these reprints before the particular issue goes to press. There will be a nominal charge for reprints: $6.00 per page for the first one hundred copies, and $3.00 per page for each additional hundred.

LES SYMPOSIA PAR CORRESPONDANCE DES ACTA CYTOLOGICA

Les *Symposia par Correspondance* des ACTA CYTOLOGICA présentent des discussions internationales sur des problèmes scientifiques intéressant le cytologiste exfoliative.

Système du choix des sujets pour les symposia: En partant des propositions, et sous la rubrique: FUTURS SYMPOSIA, le bureau de rédaction dressera la liste des sujets principaux qui seront publiés dans les ACTA CYTOLOGICA.

Le bureau de rédaction établira le programme définitif et détaillé des discussions qui sera publié dans les ACTA CYTOLOGICA précédant immédiatement le symposium, sous la rubrique PROCHAIN SYMPOSIUM.

Recommandations pour les auteurs: Chaque sujet principal sera présenté par un Rapporteur Général ou des Rapporteurs. Ces mémoires principaux seront alors soumis aux Participants à la Discussion. En règle générale 600 mots seront accordés aux Rapporteurs des sujets principaux, et 200 mots aux Participants à la Discussion. Les Rapporteurs Généraux pourront clôturer les discussions par un nombre illimité de remarques.

Des microphotos et graphiques pourront être reproduits à raison d'une page entière pour chaque sujet principal et une demi page au maximum pour les discussions. Les microphotos et les graphiques doivent être présentés sur du papier brillant, de préference dans le format 12 × 18 cm. *Chaque figure devra être accompagnée d'une legende explicative précise.*

Les membres et invités prenant part aux discussions sont invités à *limiter strictement leurs interventions aux discussions des sujets principaux.* Des discussions qui n'ont pas de rapport direct avec le sujet principal *ne pourront être acceptées.* Il est recommandé que les discussions soient rédigées d'une manière telle que le lecteure aît l'impression d'assister à une discution réelle de table ronde.

Les Remarques de Clôture du Rapporteur Général devront se limiter à la réponse aux questions soulevées dans les discussions et aux autres informations éventuelles ayant un rapport direct avec le sujet.

La bibliographie des rapports et discussions devra être rédigée de la même manière que celle de l'American Journal of Obstetrics & Gynecology et figurer à *la fin* du texte. *Chaque opinion ou publication citée dans le texte doit avoir sa réference dans la bibliographie.*

Dates limite pour les collaborations: Le bureau de rédaction, fixera des dates limites comprenaet:

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2. un délai pour les sujets principaux,
3. un délai pour les discussions,
4. un délai pour les remarques de clôture.

Tirés-à-part: les auteurs pourront obtenir des tirés-à-part de leurs communications en les demandant avant la mise sous presse des ACTA CYTOLOGICA publiant leurs articles. Les tirés-à-part seront facturés: $6.00 par page de texte pour le premier cent et $3.00 pour chaque centaine supplémentaire.

DIE SCHRIFTLICHEN SYMPOSIEN DER ACTA CYTOLOGICA

Die schriftlichen Symposien der ACTA CYTOLOGICA befassen sich auf internationaler Basis mit wissenschaftlichen Problemen, die für den Exfoliativ-Zytologen von Interesse sind.

System der Thema-Auswahl für die Symposien: Die Schriftleitung stellt auf Grund von Thema-Vorschlägen eine Liste von Haupt-Themen zusammen, und gibt diese Liste unter dem Titel ZUKÜNFTIGE SYMPOSIEN bekannt.

Die Schriftleitung bereitet das Programm mit allen Einzelpunkten vor, und veröffentlicht dieses Programm in dem Heft, das dem betreffenden Symposion vorausgeht, unter dem Titel DAS NÄCHSTE SYMPOSION.

Instruktionen fur Autoren: Jedes Thema wird von einem oder mehreren Referenten behandelt. Diese Referate werden dann von Diskussions-Vortragenden besprochen. Im allgemeinen werden Referate auf etwa 600 Worte beschränkt, und Diskussions-Vorträge auf 200 Worte. Die Referenten erhalten dann die Gelegenheit, Schlussbemerkungen ohne Wortzahlbeschränkung zu machen.

Mikrophotographien und Tabellen können abgedruckt werden: eine Ganzseite kann Referenten und eine halbe Seite Diskussionsvortragenden für Abbildungen zur Verfügung gestellt werden. Die Photographien sind auf Hochglanzpapier, und möglichst in der Grösse 12 × 18 cm erbeten und soll ein proportionales 10μ Zeichen auf der Rückseite haben. *Jede Abbildung muss von einem erklärenden Untertitel begleitet sein.*

Die Diskussionsvortragenden sind gebeten, sich in ihren Beiträgen *streng an das Hauptreferat zu halten.* Diskussionsbeiträge, die sich nicht an das Hauptthema halten, *können nicht berücksichtigt werden.* Es wird vorgeschlagen, dass die Diskussionsvorträge in einem Stil abgefasst sind, dass der Leser den Eindruck gewinnt, als ob es sich um eine Diskussion am runden Tisch gehandelt hätte.

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Die Bibliographie der Referate und der Diskussions-Vortäge sollen *am Schluss* der Beiträge nach dem Muster der Bibliographien im American Journal of Obstetrics and Gynecology aufgeführt werden. *Jede zitierte Ansicht oder Publikation muss eine Referenz in der Bibliographie haben.*

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2. Termin für Erhalt der Hauptreferate,
3. Termin für Erhalt der Diskussions-beiträge.
4. Termin für Erhalt der Schlussbemerkungen.

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El simposium escrito de ACTA CYTOLOGICA contiene discusiones internacionales sobre problemas científicos que son de interés para el citólogo exfoliativo.

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1°. Fecha límite para acuerdo de contribución,
2°. Fecha límite para las ponencias,
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DEFINITION, MORPHOLOGY, CYTOCHEMISTRY AND DIAGNOSTIC IMPORTANCE OF SPINDLE-SHAPED SQUAMOID CELLS (SNAKE CELLS, FIBER CELLS, SPINDLE CELLS)

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Symposium A

DEFINITION, MORPHOLOGY, CYTOCHEMISTRY, AND DIAGNOSTIC IMPORTANCE OF SPINDLE-SHAPED SQUAMOID CELLS (SNAKE CELLS, FIBER CELLS, SPINDLE CELLS)

DEFINITION OF SPINDLE-SHAPED SQUAMOID CELLS

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

GEORGE N. PAPANICOLAOU
New York, New York, U.S.A.

J. PAUL PUNDEL
Luxembourg, Luxembourg

JAMES W. REAGAN
Cleveland, Ohio, U.S.A.

GEORGE L. WIED
Chicago, Illinois, U.S.A.

The spindle-shaped squamoid cell ("spindle cell," "fiber cell" or "snake cell") has an elongated fusiform configuration. The term "fiber cell" or "snake cell" is used to describe the long form while the term "spindle cell" implies a shorter form having greater width.

Spindle cells can be observed in aspirations of the posterior vaginal fornix, in aspirations of the uterine cervix, and in scrapings from the cervix. The cells may be isolated or arranged in sheets. When isolated the cell outline is usually distinct while in a sheet-like arrangement the cell outlines are ill-defined.

With the Papanicolaou or Shorr stain the cytoplasm stains orange or red and in some instances is blue or green. The length of the cell must be more than three times its width and is usually 5 to 20 times its width. The nucleus generally is single and located at or near the middle of the cell. There is usually one nucleus which is regular in shape and often hyperchromatic, without discernible cytoplasm between the nucleus and the lateral cell borders.

The spindle cell is to be distinguished from the "tadpole cell" or caudate cell, which has the nucleus located at one end of the cell, and from pseudo spindle cells, which are rolled-up squamous cells.

DISCUSSION

GEORGE N. PAPANICOLAOU, New York, New York, U.S.A.

Cells exfoliating from the superficial layers of the epithelium of the cervix often assume elongated forms, which, if extreme, may cause them to resemble fibrous cells. Because of this resemblance they are more commonly described as "fiber cells" or "snake cells." Cells exhibiting such an extreme elongation are often seen in epidermoid carcinomas of the uterine cervix and some other organs, such as the lung. Their definition may thus be briefly formulated as follows:

A "fiber cell" or "snake cell" is an abnormal epithelial cell with an extremely elongated form and an atypical, also elongated, and pyknotic nucleus, frequently observed in the exfoliated state in cervical as well as in epidermoid carcinomas of other organs.

Cells of this type may be of either ectocervical or endocervical origin and represent a markedly aberrant form. Their staining reaction depends largely on the extent of their cytoplasmic differentiation. When stained with the standard OG-EA staining procedure, they show a preference for eosin or Orange G, though some cells stain greenish or greenish-blue, which apparently indicates a lesser degree of cytoplasmic differentiation.

The length of the cells is usually 5-20 times their width. Their outlines are well defined.

The nucleus is almost invariably single and has an elongated or spindle-like form. It is usually situated in the middle of the cell in close contact with its lateral cell borders. The cytoplasmic mass is thus nearly equally distributed between each end of the nucleus and that of the cell. Both cells and nuclei are usually tapered at their ends.

The nucleus is generally single and is deeply stained with hematoxylin or other nuclear stains. Its hyperchromatic condition is chiefly due to densification, which also accounts for the usual lack of discernible structural details.

"Fiber" or "snake" cells appear singly or in sheets consisting of several layers of cells. When in sheets they are usually arranged in a fairly regular pattern, and their nuclei show a relative uniformity in size, shape and structure, a feature rarely seen in malignant tumor cells.

Other types of elongated cells found in vaginal and cervical smears are the "spindle cells," which have a typical spindle-like form and the "tadpole cells," which assume the form of a tadpole. Such types are not infrequently encountered in squamous cell carcinomas of the cervix, but their diagnostic significance depends much less on their form than on the structural characteristics of their nuclei. "Spindle," "tadpole," or other types of elongated cells with normal looking oval or round nuclei should be interpreted as atypical non-malignant forms.

Connective tissue cells may occasionally be seen in smears from certain organs, but only rarely in those prepared from the vaginal and cervical secretions. Their wavy pattern and their basophilic staining reaction are some of the criteria by which they may be distinguished from the "fiber" or "snake" cells.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

Spindle cells can originate from different places:

a) They can be derived from vaginal or cervical epidermoid epithelium.
b) They can be derived from the glandular epithelium of the endocervical canal.
c) They can be derived from the connective tissue underlying the epithelium of the cervix or vagina.

Only the epidermoid spindle cell stains orange or red (if it represents a differentiated cell form) while the glandular and connective tissue cells of spindle shape always have a cyanophilic cytoplasm.

In the glandular and the connective tissue spindle-like cells, the nucleus often conserves its normal structure and is not too hyperchromatic. The cytoplasm of the connective tissue cells usually has a wavy pattern. These connective tissue spindle-like cells should not be considered as true spindle cells, for practical purposes, if they can be clearly defined as connective tissue in origin. But if the cells are single, it is not always possible to make a precise differential diagnosis concerning their origin since the differences between spindle-like cells of various origins are not typical in all cases.

For general cytological terminology, it would be preferable to apply the term "spindle cell" only to those cells arising from an epidermoid or glandular epithelium or from epithelial cells in general. As spindle cells almost never arise from a normal epithelium, they can be considered as abnormal cells, either benign or malignant. Benign spindle cells are frequently observed in the vaginal smears of older women showing a marked atrophy of the vagina, and especially after complete hysterectomy. As for those spindle cells of which the squamous epithelial origin is beyond doubt, I have not as yet been able to find in many vaginal biopsies histological pictures permitting the conclusion that these spindle cells exist as such in the original epithelium. I would believe that these spindle cells are artifacts. They probably assume the spindle form while being smeared on the slide, because these cells, coming from an atrophic and nearly always infected vagina, have lost the normal consistency of the cell membrane or cytoplasm. In biopsy material I have found the spindle cell form in the histological picture only in cases of marked dysplasia of the cervix, frequently associated with marked epidermization, and in cancer cases. In these cases, spindle cells appear in the smear usually as single cells and not in sheets. One could call these spindle cells true spindle cells because they exist in the spindle cell form in the original tissue, as opposed to the other form (pseudo spindle cells or artifact spindle cells?) which do not

exist in the original tissue. Furthermore, these true spindle cells have a different significance, since they arise either from a malignant or dysplastic lesion. But the differential diagnosis between these two types in the smear seems to be very difficult. I would like to have the opinion of other cytologists about this problem.

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

I am a little surprised that Pundel feels that spindle cells occur as such in tissue only rarely. I certainly don't feel that they are an artifact of smearing the secretion. Isn't it much more likely that since these cells are so long and thin, the possibility of cutting them their entire length to include the nucleus is extremely unlikely. Years ago when we were doing fresh impressions of tumors we saw fiber cells.

JAMES W. REAGAN, Cleveland, Ohio, U.S.A.:

I am in agreement with Ruth Graham that these cells do have a counterpart in tissues.

While cells having this configuration may be numerous in the presence of cancer, they are by no means pathognomonic of cancer. Of all the malignant tumors in which cells have been studied to date, the keratinizing carcinoma characterized by the presence of epithelial pearls is more likely to shed cells of this type. This holds true for both the experimental animal and the human. Similarly, in the human a keratinizing cancer occurring in cervix, urinary bladder, lung, skin, bone, lymph node or elsewhere is usually accompanied by these cell forms. The configuration of the cell is probably not artifactual. In many instances with suitable staining methods a delicate longitudinal fibril can be demonstrated in the cytoplasm of the more elongate forms and also in the caudate forms. This is probably a remnant of the fibrillar apparatus which is now believed to be related to the process of keratinization. This cell, therefore, may be indicative of abnormal keratinization, which may occur in some types of cancer.

GEORGE L. WIED, Chicago, Illinois, U.S.A.:

The title of our subject, DEFINITION OF THE SPINDLE-SHAPED SQUAMOID CELL, actually excludes the non-squamoid cells which have "spindle shape," such as the rarely occurring cells from the connective tissue (Fig. 1) and the cells from the glandular epithelium (Fig. 2). Photomicrographs of these cell types are shown here in order to demonstrate what we are not concerned with in this symposium, especially since the cytological differentiation of these cells generally offers no major difficulties.

In the group of squamoid cells with spindle shape we distinguish those which are obviously artifacts (Fig. 3 and 4) from those which seem to be shed originally in this spindle-like shape. I do feel rather strongly, however, that the spindle-shaped squamoid cells (whether they are called spindle cells, fiber cells or snake cells) are not mere artifacts. One can observe the same features in unfixed and unstained smears under the phasemicroscope.

I would agree with Dr. Reagan that the occurrence of spindle-shaped squamoid cells is not necessarily pathognomonic for carcinoma. However, I would be inclined to believe that if one can differentiate these cells from non-squamoid cells with spindle shape and from artifacts, which does not seem too difficult, these cells come as close to being pathognomonic for squamous carcinoma as we can come with individual exfoliated cells.

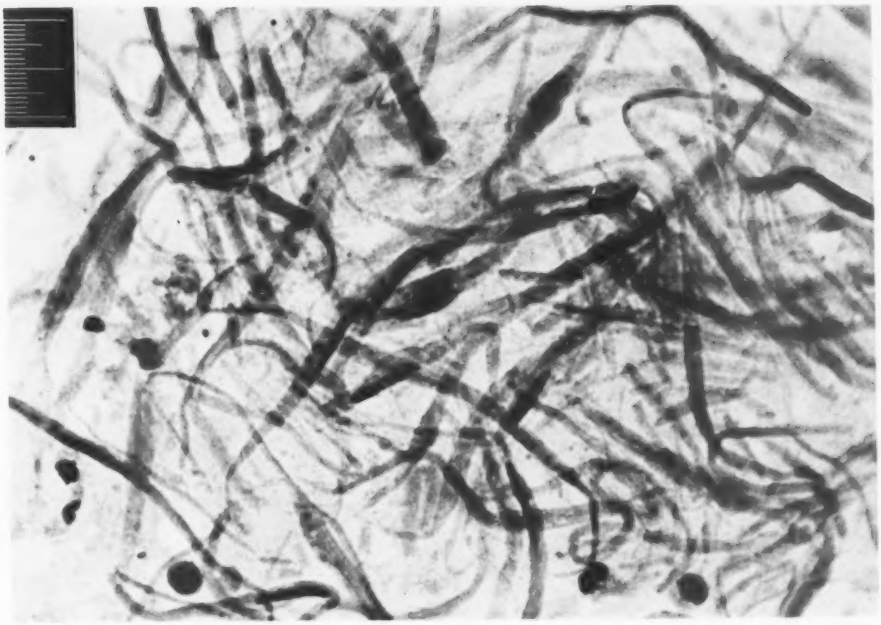

Fig. 1. Fibroblasts. Vaginal smear of a 45-year-old patient with degenerating uterine myoma. (G. L. Wied).

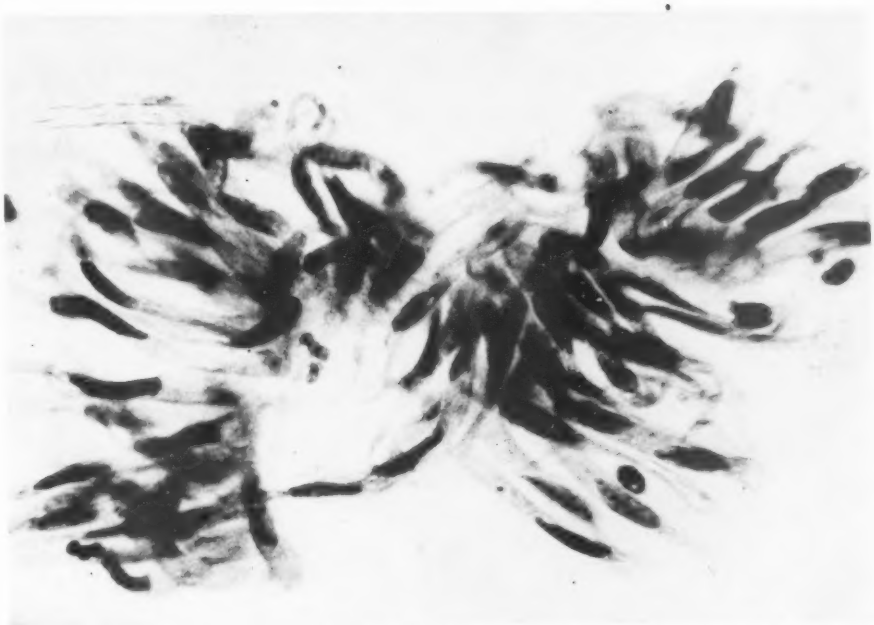

Fig. 2. Columnar cells. Elongated glandular cells in an endocervical smear of a 27-year-old woman with normal gynecological findings. (This photograph was contributed by H. Werner Boschann.)

Fig. 3. Normal intermediate squamous cells. Rolled intermediate squamous cells giving the impression of elongated cells. (This photograph was contributed by H. Werner Boschann.)

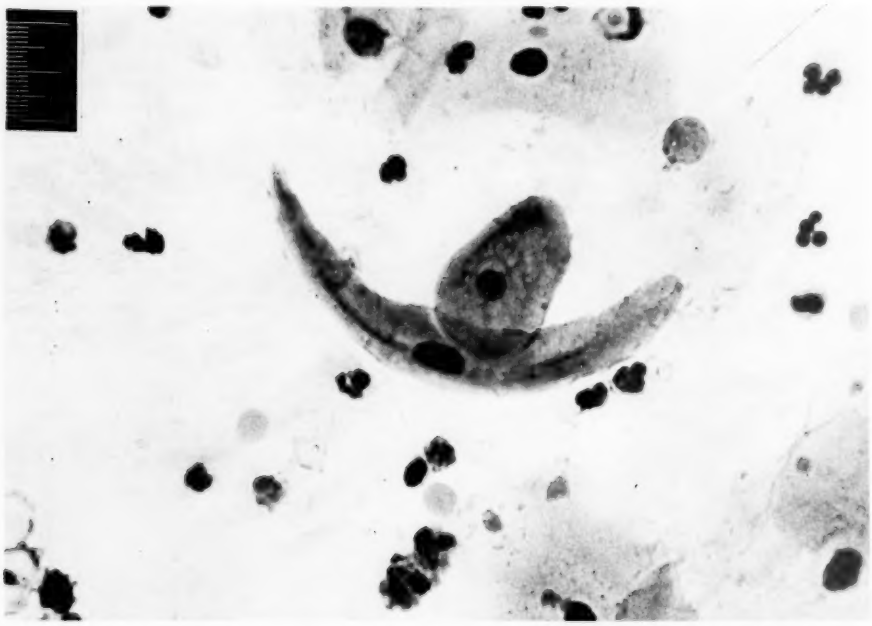

Fig. 4. Slightly atypical squamous cells. In the original slide those two cells in the center of the photograph stained deeply eosinophilic. Cervical smear of a 26-year-old patient with epidermization of cervical glands. (G. L. Wied).

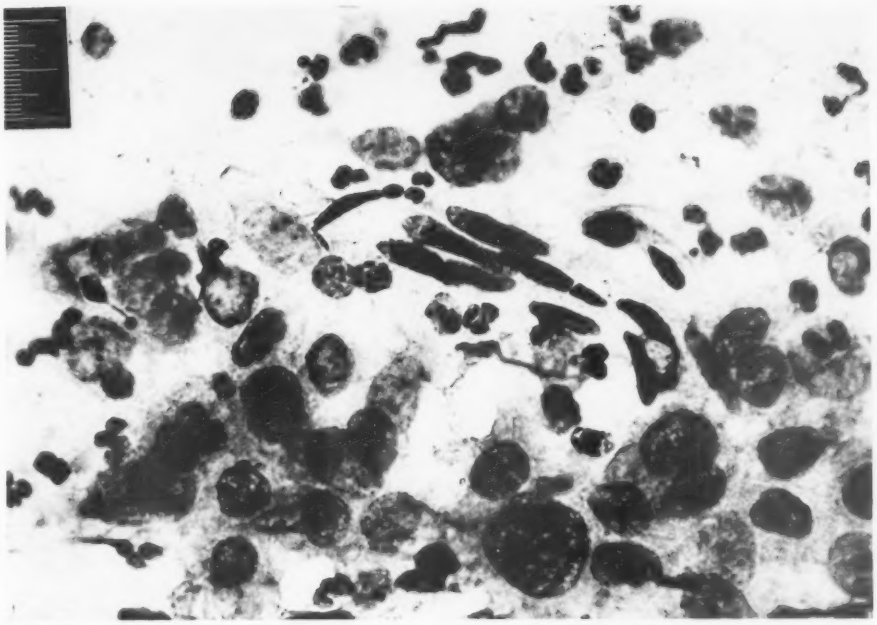

Fig. 5. Spindle-shaped squamoid cells and other tumor cells. Cervical smear of a 30-year-old patient with early invasive squamous carcinoma. (G. L. Wied).

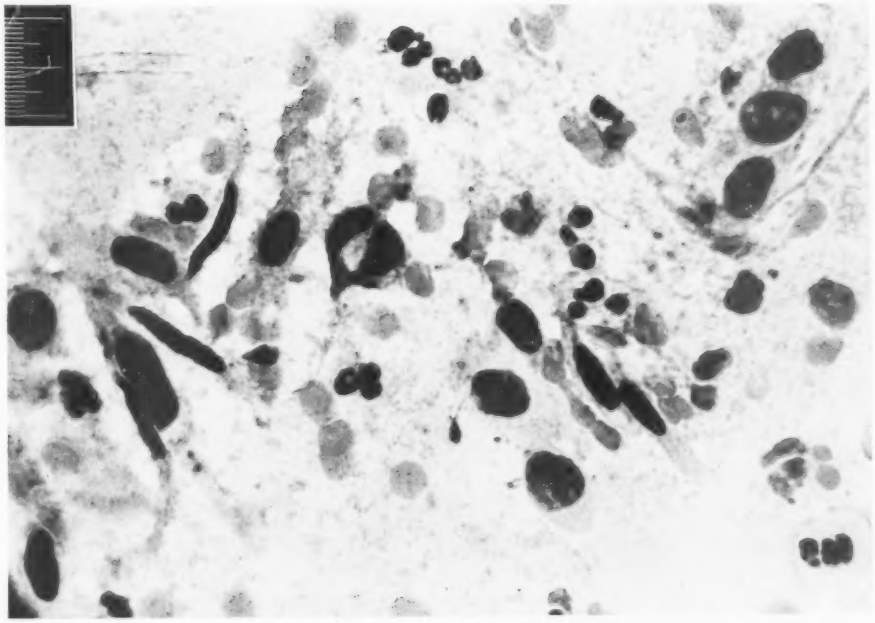

Fig. 6. Spindle-shaped squamoid cells, in the same case as in Fig. 5. (G. L. Wied).

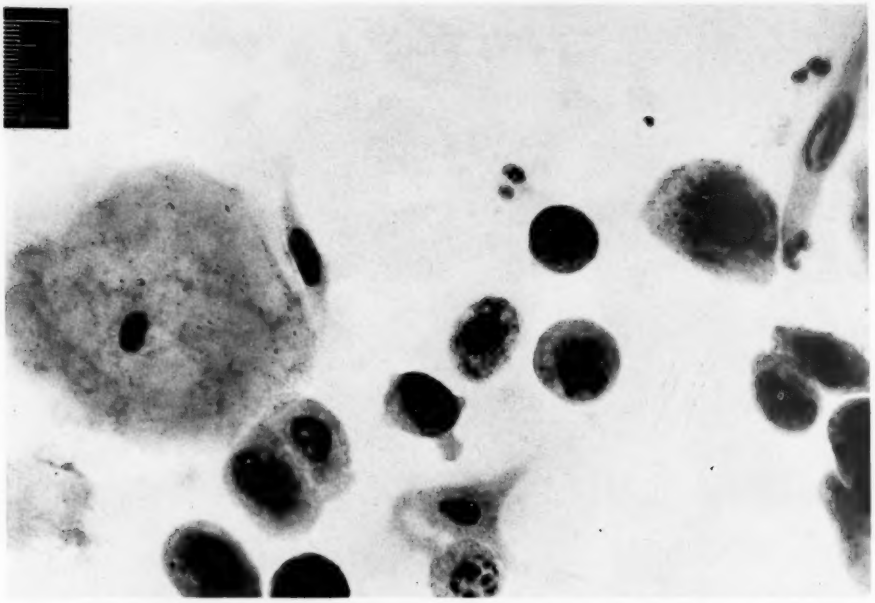

Fig. 7. Two spindle-shaped squamoid cells, a normal large cell and small cells (parabasal cell dyskaryosis). Cervical smear of a 28-year-old patient with carcinoma in situ which involves cervical and endocervical glands. (G. L. Wied).

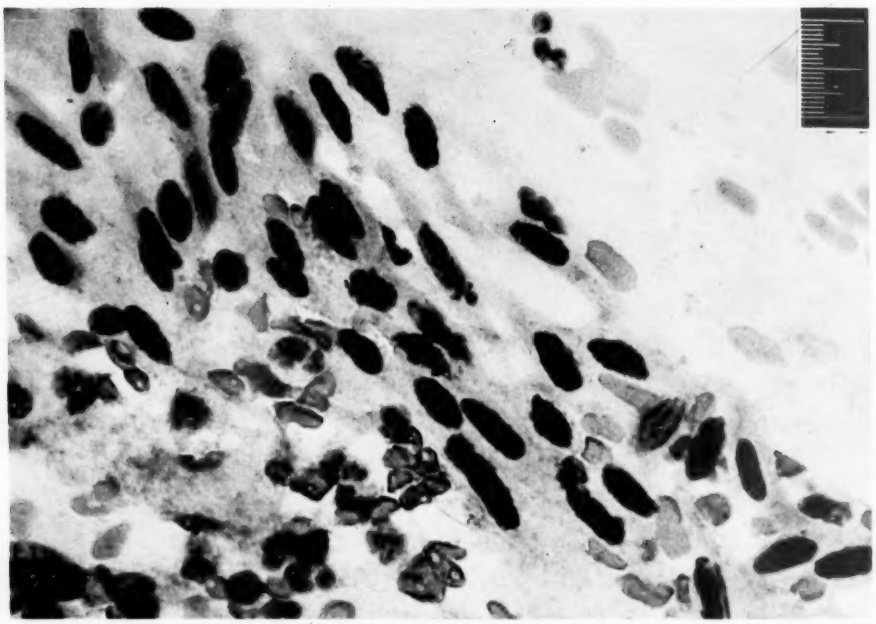

Fig. 8. Cluster of more or less degenerated spindle-shaped squamoid cells, in a vaginal smear of a patient with invasive squamous carcinoma of the uterine cervix. (G. L. Wied).

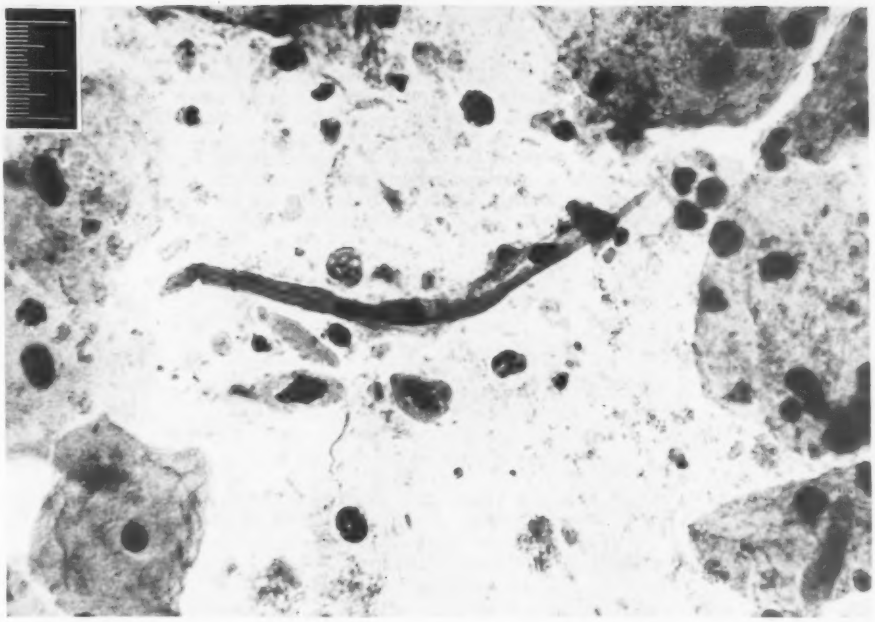

Fig. 9. Spindle-shaped squamoid cell (snake cell) and other small tumor cells in a patient with invasive squamous carcinoma. (G. L. Wied).

Fig. 10. Spindle-shaped squamoid cell (snake cell) in the same case as in Fig. 9. (G. L. Wied).

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

The terms "spindle cell" and "fiber cell" are used to indicate a malignant cell desquamating from a squamous carcinoma. It is important that we do not use the same terms for malignant and benign cells. I suggest that we use the term fibroblast rather than "connective tissue spindle-like cell." If squamous cells considered benign assume an elongated form, "aberrant basal cells" would be an appropriate term indicating the cells had an unusual form but were considered benign. "Rolled superficial cell" is more precise than "pseudo spindle cell." Since endocervical cells are elongated normally, it seems redundant to call them "glandular spindle-like cells." If we are to make any progress in terminology, we must use precise terms and avoid using the same terms for malignant and benign cells and then trying to sort them out by adding a series of modifying adjectives.

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MORPHOLOGY OF SPINDLE-SHAPED SQUAMOID CELLS

PETER STOLL
Heidelberg, Germany

The following cells with spindle shape are distinguished:

1. spindle-shaped squamoid cells in mature squamous carcinoma,
2. spindle-shaped squamoid cells in benign squamous metaplasia,
3. endocervical spindle-shaped columnar cells,
4. mesenchymal spindle-shaped cells (fibrocytes, fibroblasts), and
5. artifacts, e. g. degenerated leukocytes.

1. Spindle-shaped squamoid cells are found in mature squamous carcinomas with mono-cellular cornification and formation of horn pearls. The cytoplasm of these cells possesses a high degree of maturation (redifferentiation by von Albertini) and attempts to imitate the function of the surface epithelium in monstrous form.

In this group one may distinguish:

(a) completely mature cells (eosinophilic cytoplasm after staining with the Papanicolaou technique)

(b) relatively mature cells (cyanophilic cytoplasm).

The form of the cells is a blunt spindle shape. The cell as a whole appears plump. The length of the cell is more than six times larger than the diameter of the basal cell. In its center the width of the spindle-shaped squamoid cell may be as large as the basal cell. The cell is, however, not flattened, thus exhibiting the same thickness if observed from above as from the side. The term "red-shaped with sharpened ends" would be more suitable than "spindle-shaped."

The nucleus is hyperchromatic, usually structureless. The nucleus generally fills the entire cell at the center and laterally touches both cellular membranes. In mature spindle-shaped cells the chromatin is so dense that the nucleolus is no longer discernible.

Cases of marked nuclear condensation are referred to as karyopyknotic; karyopyknosis occurs in a nucleus of large mass. Despite this condensation the nucleus can be three times the size of nuclei of normal basal cells. One may occasionally find several nuclei in one cell, all of them exhibiting the properties of the malignant nucleus. It seems that the form and the size of the spindle-shaped squamoid cells is rather uniform in an individual case, but the one may vary considerably if one compares smears of different patients.

2. One may find spindle-shaped squamoid cells in metaplastic lesions, too. In those cases the cytoplasm appears eosinophilic and the nucleus, pyknotic; the ends of the cell are finely extended and filiformly wavy.

3. The structure of the endocervical columnar cells, which are of elongated shape, appears much finer as compared to the structure of malignant spindle-shaped squamoid cells. The ends of the columnar cells are often sharply pointed. Their cytoplasm stains blue and contains very fine vacuoles. It is characteristic for these cells to lie in palisade form.

4. Mesenchymal cells of true erosions or from an ulcer are even finer and usually as short as normal basal cells. Their nuclei are clublike, usually sharply pointed on one end, dark and without any discernible structure. These cells are characterized by their fineness and can be usually differentiated easily from the other cell types.

May I propose the following terminology:

1. Spindle-shaped squamoid carcinoma cell.
2. Filiform squamous epithelial cell.
3. Filiform columnar cell.
4. Fibrocyte (filiform).

DISCUSSION

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

I agree completely with Stoll in the morphology of the spindle-shaped cancer cell in mature squamous carcinoma. The concept that these cells "attempt to imitate the function of the surface epithelium in monstrous form" is a good one. The fiber cell, a term I prefer to spindle-shaped, is unique in my experience in that it is the only malignant cell which can be identified readily which has a pyknotic nucleus rather than chromatin irregularity. It appears to imitate the maturation of a superficial squamous cell even though its shape is abnormal and its nucleus large.

The second point is confusing to me. In the discussion of spindle-shaped cells in squamous carcinoma, these cells are referred to in (a) as cells with eosinophilic cytoplasm, and also as having nuclei which are karyopyknotic. Under number (2), the spindle-shaped cells of metaplasia are described as being eosinophilic and having a pyknotic nucleus. How then does one distinguish between these two cells? I would appreciate comments concerning this point.

If Stoll does recognize such cells and can suspect metaplasia before a biopsy is taken, I would be very interested to know exactly how he distinguishes them from the malignant cells of spindle-shape. He suggests that these cells are thinner by describing them as wavy and filiform. Is the cytoplasm the distinguishing characteristic?

I agree that cells which appear elongated are present in benign conditions though we have not been able to correlate their presence with metaplasia. I am assuming that Stoll means squamous metaplasia. We identify such cells as benign by their small, contracted, pyknotic nuclei. These benign cells only differ from the normal in that their shape is aberrant. We have preferred to call them aberrant squamous cells, rather than use a term already in our opinion reserved and restricted for a certain kind of malignant cell.

As I indicated in the discussion of the definition of the malignant fiber or spindle cell, I see no need to add adjectives such as spindle, funiform, or elongated to the columnar cell. The word column suggests elongation. However, if it is felt that columnar cells and malignant spindle or fiber cells may be confused, it should be pointed out that the distinguishing characteristic is the smooth, benign granularity of the columnar cell nucleus.

Fibroblasts are easily distinguished by their benign granular nucleus, their thread-like appearance and wavy cytoplasm. These cells are generally known as fibroblasts in this country. Is fibrocyte the term used in German?

May I again make the plea that we do not use the same term for malignant cells as we do for normal cells. The terms spindle cell and fiber cell should be reserved for differentiated cells of squamous carcinoma.

WOLFGANG KORTE, Bonn, Germany:

One can complete the excellent presentation by Stoll in the following way:

1. Spindle cells, the malignant type, coming from the epithelium, will be found in so-called carcinoma "solidum colli," as well as in mature squamous carcinoma. They are, in general, less wide, usually drawn into long shapes. The nuclei are relatively larger, the cytoplasm bright, but less matured than the forms described under (b) as relatively matured. While spindle cells of mature, keratinized squamous carcinoma often show their saucer-like, half-moon shapes in smears (one can find pearl-like layers of spindle cells in the smear), the exfoliated spindle cells from carcinoma "solidum colli" are similar to the spindle cells in their original form. That is, they are almost always stretched straight out, perhaps folding their drawn-out ends by lack of turgor, in some cases.

2. Although the dense nuclear chromatin is a characteristic of hyperplastic (and neoplastic) cells, I believe, as does Stoll, that one may speak of the nuclei from the spindle cells of mature squamous carcinoma as a "pyknosis of originally large, chromatin-rich nuclei." This probably would not occur if the course of growth of the tumor were not directed downward so that the superficial cells of the imitated epithelial layer can be exfoliated without decomposition of the carcinoma. In such a way, however, the lack of other choice keeps the cells in the center re-

spective to the axis of the tumor under the growth "outburst" of the basally situated tumor cells, as they have to manage with the minimal existing space, thus extending themselves into concentric layers and becoming relatively pyknotic.

3. The appearance of several nuclei in a cell, all with qualities of a "malignant nucleus," can be due to the fact that in addition to atypical mitosis forms (indirect division) in malignant tumors, frequently the (normally rare) direct nuclear division (mostly without division of the cytoplasm) also occurs. Thus, nuclei without normal or atypical chromosome division result.

4. Spindle cells which are derived from mesenchyme, connective tissue substance or muscle occur--although rarely--in smears as "malignant" forms in so-called mucoid sarcoma, which is derived from the interstitium of the endometrium and is not rarely a spindle cell sarcoma. The cell form here is generally essentially smaller than that of epithelial spindle cells. The same is true for the spindle cell myosarcoma which has broken into the cavum uteri.

5. In regard to the discussed spindle cell forms from uterine corporal areas and from sarcoma, which might be discussed later, I would like to suggest that we consider the following nomenclature for the main groups:

a) epithelial neoplastic spindle cells
b) mesenchyme neoplastic spindle cells
c) epithelial non-malignant spindle cells
d) histiogenic cells (fibrocytes).

HANS KLAUS ZINSER, Cologne, Germany:

Stoll's grouping of the spindle cells under four headings I find very suitable. One should not, however, put the artificially elongated leukocytes (No. 5) into this classification, since one deals here mostly with degenerative phenomena of the nuclei. One will have to distinguish in the first group the differentiated spindle cells from immature types. While the cells in the first group imitate, in their morphological structures, the features of the superficial cells, the cells in the second class show cytoplasmic features of dedifferentiation resulting in the occurrence of extended cellular forms. Such cell forms are very variable in their size. The larger forms present themselves as bizarre, snake-like cells; the small ones are the fiber cells, which are similar to the fibrocytes and which are often crowded.

Spindle cells, which are found in metaplastic processes, show intercellular bridges in addition to several filiform processes. They are usually clearly distinguished from malignant spindle forms and often are called spider cells because of their special morphological properties.

It is known that cylindrical cells also can often exhibit spindle forms. Frequently one recognized, however, only one tapering cytoplasmic process, in which one finds the darkly stained nucleus, while the other end of the cell has a club shape. These forms, as a rule, are undoubtedly clearly classified as derivatives of the cylinder epithelium.

Finally, one can distinguish the mesenchymal cells from the squamoid spindle-shaped cells. The fibrocyte is narrow and has a filiform structure with an elongated nucleus and its cytoplasm stains blue. Fibrocytes are often crowded.

Due to folding and curling of dyskaryotic, superficial and intermediate cells, one occasionally finds forms which simulate spindle cells. In order to support the description of the previously named particular classes of spindle-shaped cells, I refer to the figures Nos. 73, 96, 95, 101, 118, 119, 121, 122, 150, 170-175 in my monograph (1).

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CLOSING REMARKS

PETER STOLL, Heidelberg, Germany:

The scheme of Korte contains all possible kinds of spindle cells:

	Neoplastic (malignant)	Neo-neoplastic (benign)
Epithelial	squamous cell carcinoma adenocarcinoma	squamous metaplasia columnar
Mesenchymal	sarcoma	histiogenic (fibrocyst, fibroblast, angioblast)

I agree with Zinser to withdraw the artificial changes in leukocytes from this scheme.

Zinser has already answered the question of Ruth Graham in regard to spindle cells in metaplasia and how to distinguish them from malignant cells of spindle shape. Besides the intercellular bridges seen in spindle cells of metaplastic origin, the nuclei of such cells are much smaller than the nuclei in malignant spindle cells. Both are condensed and pyknotic but differ in size.

The word "columnar" does not necessarily indicate elongation. In the fluid of adenocystoma pseudomucinosum of the ovary (which is lined by columnar epithelium), one finds the free cells of this epithelium usually of a round or oval shape. The possibility which cells possess of modifying their shape outside of tissue-connection after exfoliation is a sign of the immaturity of the cytoplasm. To me, spindle-shaped columnar cells, therefore, are ripe cells which were packed together very closely in the tissue-line and were not able to change their shape after exfoliation.

In regard to fibroblasts and fibrocytes, we call fibrocytes those mesenchymal cells which do not undergo mitosis ("ruhender" fibrocyte). They can change into fibroblasts in case of proliferation of the connective tissue (inflammatory reaction, granulation-tissue) where there are also angioblasts present. It may be worthwhile to consider the restriction of the term "spindle cells" only to those malignant squamous cells of spindle type. I feel, however, that this would not be good and I would prefer to use the epithets: malignant epithelial spindle cell, benign histiogenic spindle cell, etc.

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CYTOCHEMISTRY OF SPINDLE-SHAPED SQUAMOID CELLS

HANNS-WERNER BOSCHANN
West-Berlin, Germany

The following cytochemical observations on spindle-shaped squamoid cells are based on experiences gained from comparative cytochemical examinations of exfoliated cells from 73 cases with carcinoma in situ and 73 cases with invasive cervical carcinoma. The technique has been shown in the table of cytochemical techniques in a previous publication (H. W. Boschann, ACTA CYTOLOGICA 1: 30-33, 1957).

I. CYTOPLASM

Desoxyribonucleic Acid (DNA): negative results with the Feulgen and Methyl Green reactions.
Ribonucleic Acid (RNA): slight to negative basophilia, always negative after ribonuclease treatment (Brachet-Test). Especially with supravital procedure, the cytoplasm stains with Pyronin, even when there is no more staining reaction found with Toluidine Blue (unfixed cells, suspended in Ringer solution, Unna-mixture modified according to Ayre).

Polysaccharides: markedly positive Periodic Acid-Schiff reaction, also after previous treatment with diastase and hyaluronidase. No metachromasia after Toluidine Blue. One apparently deals here with a proteo-bound glycogen or mucopolysaccharide, respectively.

SH-Groups (Pre-Keratin): the cytoplasm of the most mature cell forms (which appear red or orange with the Papanicolaou staining procedure) stain deep blue with the Ferri-ferricyanide reaction of Frédéric and Chevremont. Berlin Blue is formed as a sign of strongly reducing SH-groups, as they are found in cysteine, the characteristic constituent of pre-keratin.

In less mature spindle-shaped squamoid cells (which stain light blue with the Papanicolaou method), one finds a slight green staining: Prussian Green as sign of weakly reducing groups, as they occur usually in the tissue, possibly also by absorption of traces of Prussian Green which may be present in Ferri-ferricyanide solutions.

After inhibition of the activity of the sulphydryl groups by treatment with saturated watery $HgCl_2$-solution (30 minutes), the Ferri-ferricyanide reaction is negative in the spindle-shaped squamoid cells. Similar results may be obtained with the technically more complicated method of Bennett (2), which is based on the reduction of mercaptanes by SH-groups.

SS-Groups (Keratin): The Performic-Acid-Schiff reaction by Pearse (3) (which is based on selective oxidation of cystine with performic acid) stains the cytoplasm of the most mature spindle-shaped squamoid cells red (according to their nuclear structure). This constitutes a positive proof of the presence of disulfide groups, which is characteristic for cystine and for keratin. With this reaction one finds that the most mature spindle-shaped squamoid cells react as the cells in pearls in squamous carcinoma.

Alpha-Amino-Acids (Proof for Protein): Ninhydrin-Schiff reaction and Alloxan-Schiff reaction (4) give a pale pink staining reaction. For practical purposes, this reaction is specific for proteins (5), which can be demonstrated by pre-treatment with pepsin.

Lipids: Sudan Black B reaction (neutral lipids): negative.
Nile Blue Sulphate (acid lipids) and Acid Hematein method Baker (phospholipids): pale, transparent blue staining.
These reactions are not strictly specific.

Enzymes:

(a) Alkaline Phospho-Mono-Esterase: slight brownish staining of the cytoplasm with the Gomori method.

Possible explanations: partly non-specific by absorption of the calcium phosphate which is contained in the incubation solution (because the reaction is still present in lesser degrees after inactivation of the enzyme with Lugol's solution, whereas the nuclei appear as light areas left free); partly by the participation of the alkaline phosphatase in the keratin synthesis; partly by rest activity of the acid phosphatase.

(b) Acid Phospho-Mono-Esterase and Phosphamidase: similar results.

(c) Carboanhydrase: cannot be demonstrated in spindle-shaped squamoid cells or in pearls of cervical carcinoma with the method of Kurata (6) and its modification by Friederici (7).

(d) Succinic Dehydrogenase: slight activity demonstrable. Using the modifications by Rosa and Velardo (8) and Rutenburg, Wolman and Seligman (9) of the technique of Neumann (10) with tetrazolium purple, one finds the following reaction (fresh cells, pH 8.6, under a cover glass, at 37°C on heated table):

Appearance of the first formazan granules:

1. in dedifferentiated cancer cells: after approximately 5 minutes
2. in normal parabasal cells: after approximately 10-35 minutes
3. in dyskaryotic intermediate cells: after approximately 45 minutes
4. in normal intermediate cells: after approximately 45-60 minutes
5. in highly differentiated cancer cells (spindle cells, tadpole cells, round cells): after approximately 60 minutes
6. in normal superficial cells: after approximately 2-24 hours (some show no reaction).

(e) Peroxidase: negative reaction after formol fixation and 5 minutes staining in benzidine.

II. NUCLEOLUS

The nucleolus can be demonstrated only in those spindle-shaped squamoid cells in which the nucleus has not yet undergone pre-pyknotic or pyknotic changes. The nucleolus appears bright red (pyroninophilic) with the Methyl Green Pyrenine staining procedure amidst the green nuclear substances. Therefore, the nucleolus contains RNA in higher concentration, since an unstained area is left free amidst the green nuclear substance after pre-treatment with ribonuclease. The borders of this unstained area appear red (Feulgen positive) in the Feulgen reaction. The pyroninophilia is not influenced by desoxyribonuclease.

Moreover, by now one cannot demonstrate any qualitative cytochemical difference between nucleoli of normal squamous cells on one side and those of spindle-shaped squamoid and other cancer cells on the other side.

III. NUCLEUS

Nucleic Acids: The DNA-content is only seemingly increased compared with cancer cells of a lower degree of differentiation, due to the concentration of chromatin in the pyknotic nucleus:

Feulgen reaction (all polymerization stages of DNA): positive
Methyl Green (only high stages of polymerization of DNA): positive, also with vital staining
Toluidin Blue (DNA and RNA): markedly positive.

Enzymes:

(a) Alkaline Phospho-Mono-Esterase: after approximately 3 hours positive reaction (in normal cellular nuclei after 9 hours).

(b) Acid Phospho-Mono-Esterase: nuclei appear black after approximately 6 hours (nuclei of normal parabasal cells after 8 hours; nuclei of normal superficial cells after 14 hours). These results could be explained with the participation of phosphatases in the processes of nuclear growth and nuclear degeneration. The same applies for:

(c) Phosphamidase: marked activity (blackening of the nucleus after 6 hours, whereas nuclei of normal parabasal cells become black only after 8-12 hours). This marked activity is inhibited after inactivation of the enzyme (e.g. Lugol's solution).

Lipids: considerable content of phospholipid (Acid hematein method, Baker); negative reaction after pre-treatment with pyridine extraction.

The degree of reaction on nuclei of spindle-shaped squamoid cells is higher than on the nuclei of intermediate cells. Apparently this may be explained by the concentration of the nuclear substance in the pyknotic nuclei and by the nuclear degenerative processes which are connected with this pyknotic degeneration.

Thus, one finds a hyperactive nucleus which is rich in nuclear substance in a relatively mature cytoplasm which exhibits true keratinization in the most mature forms.

Qualitative cytochemical differences could not be detected between spindle-shaped squamoid cells which derived from an invasive carcinoma as compared with those derived from a carcinoma in situ.

CONCLUSIONS

Spindle-shaped squamoid cells indicate the presence of a stratum corneum. These cells are also found in lesions which otherwise show an immature structure if the surface layers are intact. They are also found in lumina of glands which are filled by ingrowing carcinoma. Spindle-shaped squamoid cells represent cytochemically, as well as morphologically, a monstrous attempt of imitation of the function of surface epithelium and show, therefore, a certain redifferentiation in the sense of von Albertini (11).

The most mature cell forms of the spindle-shaped squamoid cells exhibit in this process of redifferentiation a supra-physiologically true keratinization. Spindle-shaped squamoid cells are characteristic for a squamoid epithelium which no longer follows the normal laws of cell ripening.

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DISCUSSION

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

Boschann's papers on cytochemistry are so lucid there is really very little to discuss. I would like to ask one question. Under the section on polysaccharides it is stated that the periodic acid - Schiff reaction is still positive after treatment with diastase and hyaluronidase. Wislocki, Fawett and Dempsey (1) found that some of the normal superficial cells of the vaginal smear were also resistent to enzymatic digestion. They assumed that in some instances the cells had such heavy encapsulated cytoplasm that the enzyme could not penetrate. This was substantiated by the fact that sections taken from the vagina did not show such resistance to enzymatic digestion. They conclude that the reason some of the superficial cells remained positive was that in contrast to the sectioned cells where the interior of the cell was exposed, the cellular wall of some superficial cells does not allow the penetration of the enzyme. Would Dr. Boschann comment on whether in sections of squamous carcinoma, for instance in pearl formation, the cells are still P.A.S. positive after enzymatic digestion?

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D. G. SCARPELLI, Columbus, Ohio, U.S.A.

Boschann has performed an impressive number of histochemical reactions in his comprehensive study of spindle-shaped squamous cells. His results show that one attribute of neoplasia is to grossly exaggerate a normal cellular process, in this instance the formation of keratin.

From a purely histochemical point of view, several of the reactions and modifications employed in this study are subject to criticism. The ferricyanide method for SH-groups, though generally acceptable, is not strictly specific and should be replaced by the DDD method (1). Significant errors in the localization of enzyme activity due to spurious precipitate deposition have been repeatedly shown for the Gomori technique for alkaline phosphatase (2, 3). This reaction can be replaced by the azo dye coupling method (4). Although this too is subject to some error due to lipid solubility of the reaction product, it does give a result which is readily reproducible and consistent with biochemical data on the cellular localization of phosphatases. The same objections hold true for the phosphamidase reaction. An additional source of error is the high pH used in the succinic dehydrogenase reaction. This may result in non-enzymatic reduction of tetrazolium salt, especially during long incubation periods, by SH-groups and other intracellular reducing substances, such as ascorbic acid (5).

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CLOSING REMARKS

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

In response to Dr. Graham's question, we examined histological sections of:

1. normal vaginal epithelium, late proliferative phase,
2. carcinoma in situ,
3. invasive squamous epithelial carcinoma with pearl formation.

We used the following methods:

1. PAS stain,
2. PAS stain after 15 minutes treatment with diatase at 40°C.,
3. PAS stain after 20 minutes treatment with hyaluronidase at 40°C.,
4. PAS stain as in both methods 2 and 3.

The surface layer of the normal squamous epithelium, the spindle cell layer in carcinoma in situ and the pearls of invasive carcinoma remain PAS positive, even after treatment with diatase, hyaluronidase, and diatase plus hyaluronidase. Thus, a difference between the desquamated cells in the smear and the cells in the tissue section could not be shown.

To Dr. Scarpelli:

Since we have known Dr. Scarpelli's objections against some methods in the beginning of our studies, we have routinely performed the following controls:

In the Chevremont-Frederic reaction only the deep blue stains have been evaluated as an indicator for strongly reducing SH groups. This blue stain did not show up again after inhibition with saturated mercury chloride (30 minutes) (Lison). Under these conditions the reaction was identical with the DDD method and Bennet's method (1951), which we also used.

Alkaline phosphatase: Control sections have been put into Lugol solution for five minutes in order to inactivate the fermentation. Calcium, which was already present in tissue, was determined by discontinuation of the treatment in the substract with cobalt chloride and ammonium sulfide. There is much literature concerning the source of errors in the Gomori method. Therefore, we have considered only those results as positive which, in our own experiments, have been identical with the azo dye coupling method (Manheimer and Seligman, 1949). They agree with biochemical and physiological knowledge.

Succinic dehydrogenase: Control sections without addition of succinic acid remained negative (a) after dematuration of tissue proteins - by heating to 80°C, by alcohol or by formaldehyde, and (b) after specific inhibition of the succinic dehydrogenase with malonic acid, sodium tellurite and sodium selenite. Thus the specificity of the reactions observed has been proven according to the present cytochemical knowledge.

There is extensive cytochemical literature concerning this subject. We consider these controls compulsory, but since this space for publication is limited, we did not mention the controls separately - which necessarily leads to criticisms such as Dr. Scarpelli's. I hope to get Dr. Scarpelli's complete agreement in maintaining my original paper with the above additional remarks.

CYTOCHEMISTRY OF SPINDLE-SHAPED SQUAMOID CELLS PRIOR TO AND AFTER IRRADIATION

CONSTANTIN HEROVICI
Villejuif, Seine, France

The fiber cell is derived from the outermost layer of epithelial pearls and is characteristic of those squamoid tumors showing the highest differentiation.

We have had the opportunity of studying, to date, 60 cases of squamous carcinoma of the uterine cervix, 40 of which were highly differentiated, with numerous epithelial pearls; these cases provided us with the necessary material. Specimens obtained were both cytological, using cervical smears fixed in alcohol-ether, and histological, using biopsies fixed in an alcohol-formol-acetic mixture. In this series of 40 uterine tumors, further biopsies were obtained from 10 cases after radiotherapy.

In this paper we intend to describe fiber cells from a metabolic viewpoint when they are present in cervical smears and in cervical biopsy material, before and after irradiation. The techniques used are as follows:

(a) Morphological studies were made on smears using our I. G. R. #347 technique, which we will describe later in a separate paper (1), and on histological sections by a technique not published as yet. The latter employs a mixture of picric acid, fuchsin and aniline blue, used after a chromic hematoxylin, and shows up the maturation of collagen and keratinization of cells, as well as the usual histological features of the lesion as a whole.

(b) The study of nucleic acids was made by the Feulgen technique as well as by an individual variation of the Brachet method. This variation consists of the additon of Orange G to the methyl-pyronine green dye. It differentiates cytoplasm which is rich in ribonucleic acids (R. N. A.) and stains pink, from cytoplasm which is poor in R. N. A. and remains yellow. The background of the preparation is stained uniformly with Orange G. Specificity control has been confined to bile salts (method of Panijel) (2) or to the hydrolysis of trichloracetic acid.

(c) Intra-cellular potassium was demonstrated by Marza's modification (3) of MacCallum's technique (4).

(d) To show up those substances rich in SH-radicals, we chose the technique of Chévremont and Frédéricq (5).

(e) P. A. S. was used for polysaccharides according to Hotchkiss and MacManus (6).

(f) We used Best's Carmine to stain glycogen.

Quantitative evaluation of the results of these histological methods has been notated as follows: - to ±, +, ++, and +++.

Nucleic Acids

	Before Irradiation	After Irradiation
D. N. A. – Nucleus	+++	± to +
R. N. A. / Nucleolus	++	+++
R. N. A. \ Cytoplasm	±	++

Before radiotherapy, one can see that the nucleus of a fiber cell is rich in D. N. A., even when the majority of the other malignant cells show only minimal quantities of this nucleic acid. The distribution of D. N. A. is fairly homogeneous; often it is present in pre-pyknotic nuclei.

R. N. A. is distributed in a characteristic fashion. One sees 1 or 2 red patches of R. N. A. joined together by a very fine filamentous network, also staining red. In the cytoplasm, R. N. A. is very finely distributed and quantitatively there appears to be much less than in squamous cells from areas of active growth.

In smears taken the day after the cessation of radiotherapy, there is a definite fall in D. N. A., but this fall is less marked than in large anaplastic nuclei. Thus, in treated cancers one can see a total disappearance of D. N. A. from undifferentiated malignant cells, as opposed to persistence of this acid in fiber cells, a fact that does not seem in accordance with the prognosis of curability.

The increase in nuclear and cytoplasmic R. N. A. usually seen in malignant cells following irradiation is very noticeable in well-differentiated cells. But if the patches of ribonucleic proteins of the nucleolus increase in size and in number, one no longer sees the filaments uniting these patches. The increase of R. N. A. in cytoplasm is perhaps the most marked radio-biological reaction.

P. A. S. Reaction

	Before Irradiation	After Irradiation
Undifferentiated squamous epithelial cells	-	++
Fiber cells	++	+++

Whereas in undifferentiated malignant cells from cervical cancer the Hotchkiss and MacManus reaction (P. A. S.) (6) is negative, except in areas of growth where it is faintly positive, fiber cells appear to be the elements richest in polysaccharides. After treatment, all the neoplastic elements give a very strong P. A. S. reaction.

SH-Radical

Fiber cells exhibit a Chévremont and Frédéricq reaction (5) much more intensely than the other cellular elements, except in areas of parakeratosis. This is not surprising as we already know that pre-keratin is rich in SH-radicals. After treatment, there does not seem to be any change in these well-differentiated elements; on the contrary, the number of anaplastic cells giving a strongly positive SH reaction is much higher than before treatment.

Potassium

Although there are considerable quantitative differences in the intra-cellular potassium between one cancer and another and even between one neoplastic area and another, fiber cells are always particularly rich in this mineral. It is found in the nucleolus, in the nuclear membrane and in the cytoplasm.

After radiation therapy, all irradiated cells are rich in potassium and there are no notable differences between fiber cells and the others. If before treatment the prominence of intracellular potassium appears to be associated with a tendency to growth, we cannot think of attaching the same significance to the presence of this substance after treatment.

Glycogen

Best's Carmine stain does not seem to differentiate fiber cells from other anaplastic cells in uterine cancer.

Glycogen is absent from fiber cells before therapy and one can see traces of a positive reaction with Best's Carmine after irradiation. This occurs in all malignant cells from cervical cancer.

To summarize, after cytochemical studies of the properties of the fiber cells, we can see that this cell has definite characteristics prior to radiotherapy, but that it loses them after treatment.

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DISCUSSION

D. G. SCARPELLI, London, England:

This study clearly shows that cytochemical changes occur in fiber cells following irradiation. The marked decrease in nuclear DNA and increase in cytoplasmic RNA following irradiation is in accord with the results of other investigators (1, 2) and is indicative of altered nucleoprotein metabolism.

The post-irradiation increase in SH-groups observed in undifferentiated cancer cells is confusing in view of the fact that SH-groups are oxidized by ionizing irradiation (3). Since the specificity of the ferricyanide method for SH-groups is questionable, I would suggest that in future investigations the highly specific DDD method (4) be used. The findings with regard to potassium, though interesting, should be interpreted with caution since with present methods the localization of freely diffusible cations is fraught with difficulty and may be inaccurate.

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CLOSING REMARKS

CONSTANTIN HEROVICI, Paris-Villejuif, France:

No comments.

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PHASEMICROSCOPY ON SPINDLE-SHAPED SQUAMOID CELLS

PETER STOLL
Heidelberg, Germany

The observations of unfixed and unstained cells under the phasemicroscope permits the determination of distinct nuclear and cytoplasmic changes which are characteristic of the degree of maturation of the cells:

Nucleus (a) immature: clear karyoplasm, possibly containing a few structures, possibly with one or two nucleoli; fine and smooth nuclear membrane.

(b) increasing maturation: the nuclear structure gradually becomes more coarsely granulated until finally, with the beginning of pyknosis, the chromatin condenses, forming a dense nuclear mass.

Cytoplasm (a) immature: very fine cellular membrane, if any; few structures in the cytoplasm.

(b) increasing maturation: increasing granulation, dense cellular membrane, intercellular bridges.

The above is true for both normal and malignant cells. However, the nuclei of malignant cells remain at a low stage of maturation while the cytoplasm matures to a greater extent.

The spindle-shaped squamoid cells of squamous carcinoma are cells with a rather high degree of cytoplasmic maturation: granulation, definitive cellular membrane, intercellular bridges, rigid, unalterable cytoplasmic outline. The nucleus of the spindle-shaped squamoid cell, however, appears immature: pale, finely granulated nuclear structure, irregular nuclear membrane, mitotic activity. The nucleus becomes dense only in the stage of marked maturation with cornification of the cytoplasm.

Therefore, the carcinomatous spindle-shaped squamoid cells have the following appearance under the phasemicroscope: elongated, spindle-shaped, granulated cytoplasm with dense cellular membrane and possibly with intercellular bridges. Each cell contains a large nucleus which may show varying degrees of maturity, i.e., with few to many distinct nuclear structures. The nuclear membrane is irregular with knot-like condensation. Not infrequently one finds nuclei of different sizes. In my observation the cytoplasm remains rigid, i.e., it will not alter its form as is the case in immature cellular forms.

Summarizing: In carcinoma cells the cytoplasm and the nucleus are usually in different stages of maturation (un-coordinated maturation). In spindle-shaped squamoid cells the cytoplasm reaches the highest degree of maturation possible, whereas the nucleus may remain more or less immature.

DISCUSSION

EDMUND SCHÜLLER, Vienna, Austria

Spindle-shaped squamoid cells which derive from differentiated squamous carcinomas with cornification or tendency towards cornification are recognized under the phasemicroscope by their characteristic cellular form and the disturbed nuclear-cytoplasmic ratio. The nucleus which is rather large as compared with the cytoplasm is elongated in order to fit more or less into the spindle-shaped cell. I agree with Stoll that the nuclei show a more or less crowded and dense

chromatin structure depending on the degree of differentiation. Multinucleation is rather rare. Bizarre nuclear forms are also rare.

HANS KLAUS ZINSER, Cologne, Germany:

Dr. Stoll has pointed out that the appearance of the cells due to ripening or dedifferentiation are especially impressive under the microscope. It can be shown that differentiaion is, above all, a function of the cytoplasm. During normal maturation of the epithelial cell, the nucleus and cytoplasm pass through typical coordinated maturation stages. This relation between the nucleus and the cytoplasm is not coordinated in the atypical cells. That is, the nucleus retains all signs of immaturity, with a light nuclear content, a sharp, beaded nuclear membrane, clumps of chromatin, and a distinct nucleolus; on the other hand, the cytoplasm may follow -- in the mature neoplastic types -- the regularities of the normal differentiating cells: may develop formed structures and exhibit the cytoplasmic criteria of maturation (sharp, inflexible cell membrane with formation of tonofibril systems and dense cytoplasm granulation). In comparison, malignant spindle cells of this type have succeeded, even if not completely, in reaching the cytoplasmic structure but not the nuclear structure of the cell. In dedifferentiated malignant spindle cell forms, this differentiation or redifferentiation of the cytoplasm does not occur. It shows ameboid qualities, but a firm, distinctly formed cellular membrane and a tonofibril system do not occur. In such a way do bizarre types originate, which are completely different in size and length. The cytoplasm can be drawn into a long, snake-like shape. The tadpole cell is only a special form which becomes explicable through such processes.

There are, of course, fluid transitions between differentiated and dedifferentiated malignant spindle cells. Principally one can, however, arrange the malignant spindle-shaped cell types into the two classes named.

CLOSING REMARKS

PETER STOLL, Heidelberg, Germany:

No comments.

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ULTRAVIOLET MICROSCOPY ON SPINDLE-SHAPED SQUAMOID CELLS

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

The examination of fiber cells under ultra-violet light gives no additional information. The absorption of the nucleus is intense at 261 mμ but this could have been anticipated since the nuclei of fiber cells are often hyperchromatic. The peak of nucleoprotein absorption is at 261 mμ so that the photograph in the ultraviolet only confirms that there is increased chromatin present in the nucleus. Cytoplasmic absorption is not remarkable and differs in no way from the cytoplasmic absorption of benign squamous cells.

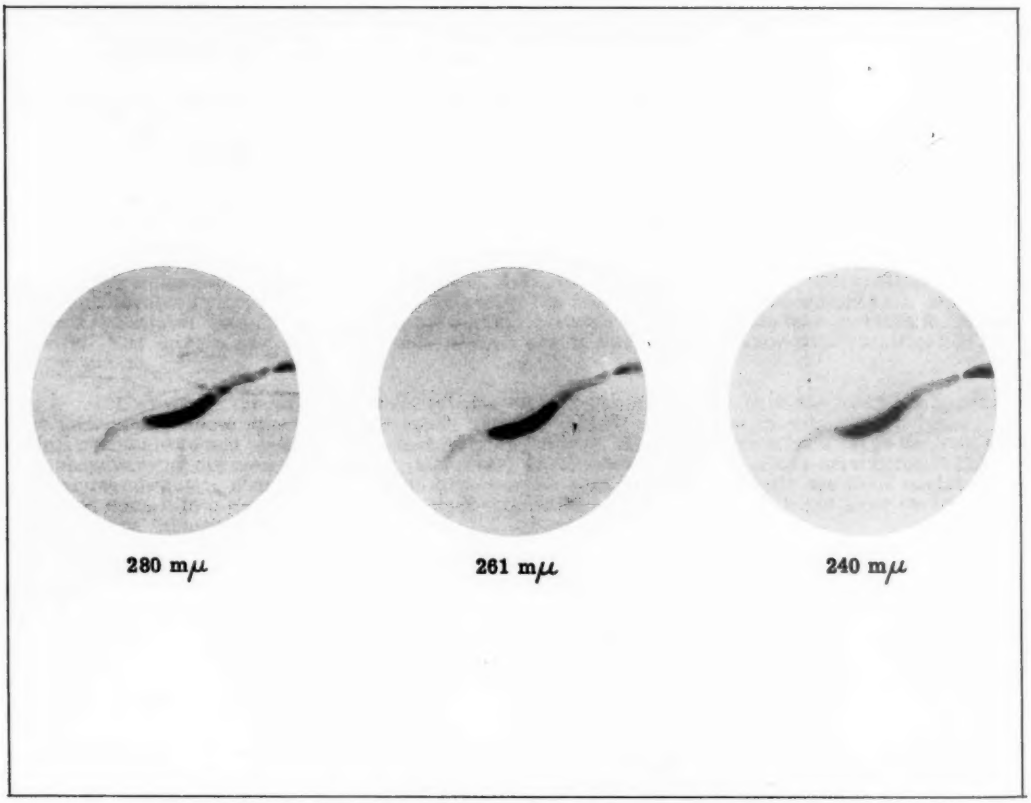

Fig. 1. Ultra-violet photomicrographs of a fiber cell at three different wave lengths.

OCCURRENCE OF SPINDLE-SHAPED SQUAMOID CELLS IN EXPERIMENTAL CERVICAL CARCINOMA

EMMERICH von HAAM

Columbus, Ohio, U.S.A.

Spindle-shaped squamoid cells have been frequently observed in our experimentally produced tumors of the cervix in mice and have always been to us a strong indication of the malignant changes taking place in the cervical epithelium (1, 2). The cells sometime appear quite early in the process of carcinogenesis and at first may not be present in every smear. We have never observed them during the stage of inflammatory changes which takes place during the first one or two months of carcinogenesis but have found them, particularly the plumper forms, already during the stage of cellular dyskaryosis. Later they increase markedly in some animals and assume very bizarre forms, while other animals fail to show any spindle-shaped squamoid cells even after they have developed large tumors. The highest number of these cells found so far has been 52 per cent in one animal with a highly malignant spindle cell carcinoma.

The appearance of spindle-shaped squamoid cells varies greatly. Some of them are small, measuring only 20 to 30 micra, while others assume huge proportions, measuring up to 240 micra in length. The cytoplasm stains more commonly acidophilic than basophilic, although the latter can also be observed. It is either homogeneous or has pale vacuoles and fibrillar structures. The plasm tapers off in threadlike elongations which sometimes may be indistinguishable from fibrin threads (Fig. 1). On the glass slide they have assumed grotesque shapes which justify the name "snake" cells (Fig. 2). The nucleus may be located in the center of the cell or at one pole. Some cells possess small pyknotic nuclei resembling the nuclei of benign cornified epithelium (Fig. 3). More frequently, however, the nucleus is large, irregular, shows a coarse chromatin structure and may possess one or two macronucleoli (Fig. 4). Binucleated cells also have been observed (Fig. 5). We feel that some of the squamoid spindle cells probably belong to the group of dyskaryotic cells and should not be classified as malignant, while others must be regarded as highly malignant cells. The difference between the two types seems qualitative rather than quantitative and there is no single characteristic sign which can be taken as an absolute criterion to differentiate the two types. The larger the nucleus the more likely they are to be cancer cells. In spite of the fact that mitotic figures are not unusual in our vaginal smears, we have never observed one in a spindle-shaped squamoid cell.

An interesting observation could be made in our experiments where the administration of the carcinogen was stopped at different intervals. Those animals which already had shown spindle-shaped cells once or twice before the carcinogen was discontinued proceeded to develop carcinoma and no spontaneous regression was observed even though the spindle-shaped cells disappeared for a short while.

We also have been able to localize these cells in tissue section of carcinoma in situ as well as invasive carcinoma. In carcinoma in situ they always occur on the surface of the lesion and seem to desquamate rather readily. In invasive tumors they appear usually in the poorly differentiated type which we have classified as spindle-cell type carcinoma. Among 72 experimental carcinomas of the cervix induced in our laboratory we have been able to observe 14, or 19.4 per cent, tumors of the spindle-cell type. In tissue sections the cells are elongated with scant, pale eosinophilic cytoplasm, large hyperchromatic nuclei and multiple macronucleoli. Numerous mitotic figures were also observed. They tended to necrotize early and invade very rapidly.

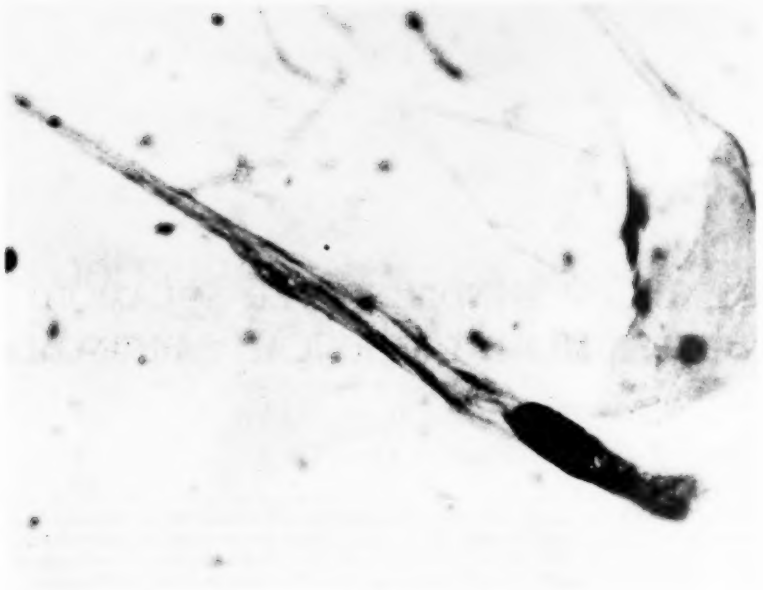

Fig. 1.

Threadlike squamoid cell found in the vaginal smear of an animal with a large invasive carcinoma of the cervix.

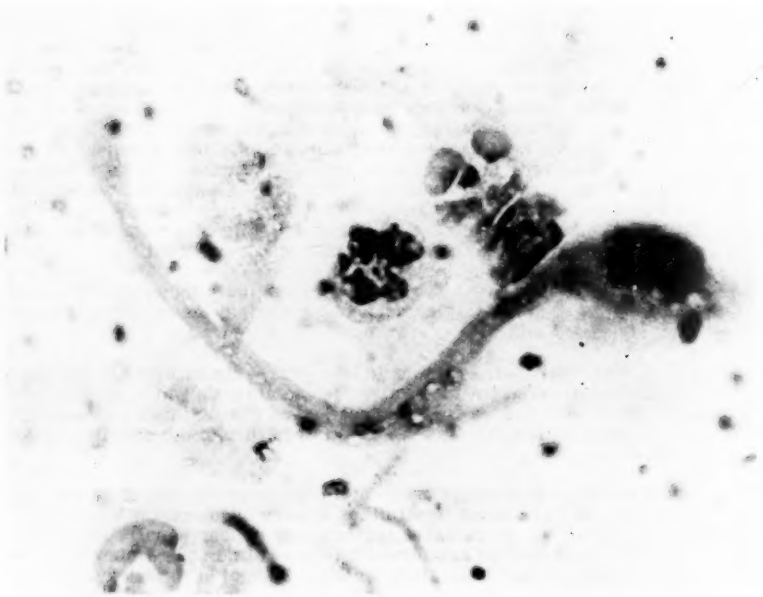

Fig. 2.

Snake cell found in the vaginal smear of an animal with a small invasive carcinoma of the cervix.

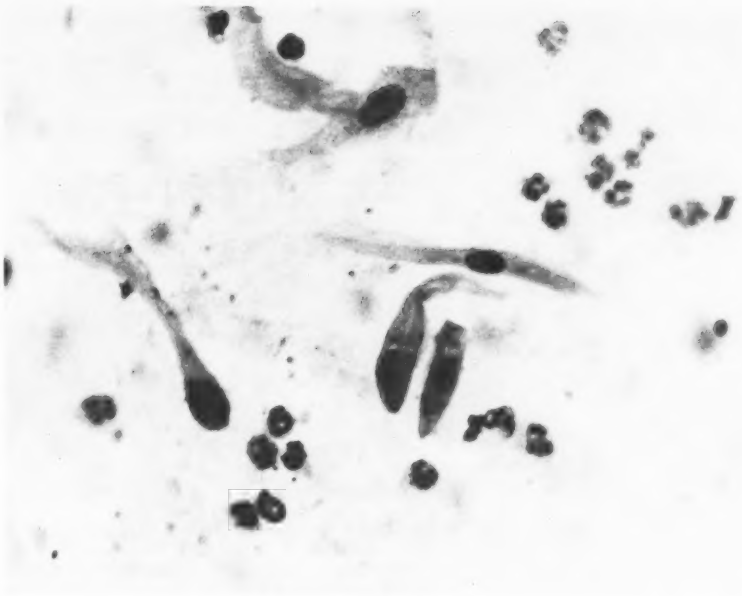

Fig. 3.

Spindle-shaped squamoid cells in the vaginal smear of a mouse with a carcinoma in situ of the cervix.

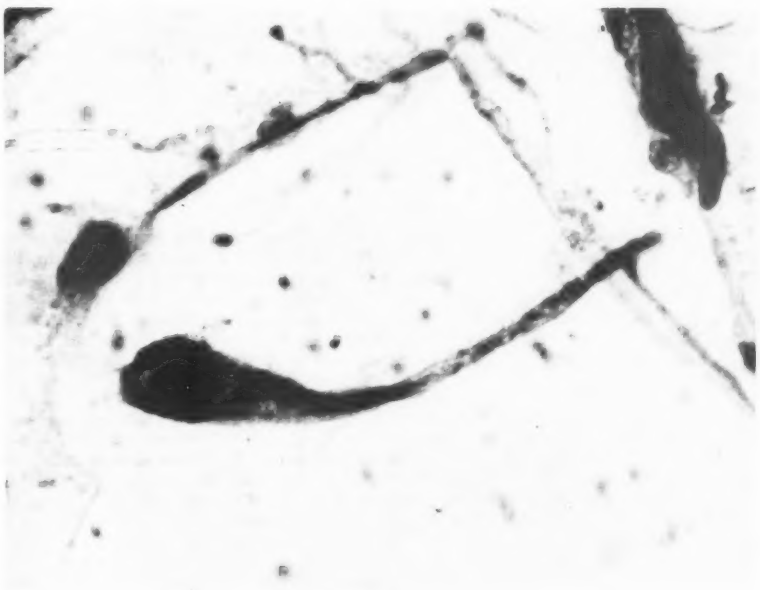

Fig. 4.

Malignant fiber cell in the vaginal smear of a mouse with carcinoma in situ of the cervix.

Fig. 5.

Malignant snake cell with two large hyperchromatic nuclei in the vaginal smear of a mouse with large invasive carcinoma of the cervix.

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DISCUSSION

JAMES W. REAGAN, Cleveland, Ohio, U.S.A.:

In studying experimental carcinogenesis in the cervix of the mouse, the presence of any significant number of spindle-shaped cells in the vaginal fluid is a strong indication that carcinoma exists. In our own work we have induced cancers of the uterine cervix by inserting a carcinogen-impregnated thread into the cervical canal[3]. This induction procedure was used successfully by Murphy. The cancers described by von Haam and Scarpelli were produced by painting the uterine cervix with a carcinogen. While the induction procedures are different the spindle-shaped cells described by von Haam are quite similar to those observed in the animals having cancers induced by the thread technique.

In our own experiences there are essentially three stages in induced carcinogenesis. The initial stage is characterized by dysplasia, in keeping with the observation of von Haam and Scarpelli. The cells desquamated into the vagina at this stage do no include spindle-shaped cells. The second stage of carcinogenesis is characterized by epithelial downgrowths into the underlying stroma. At this stage of carcinogenesis an average of 8.7 per cent of the cell forms classified in preparations from the vaginal fluid were elongate forms meeting the description of spindle cells[2]. The third stage of carcinogenesis is characterized by the presence of cancer. At this time there are spindle-shaped cells particularly those of elongate form. An average of 15.7 per cent of the cells in preparations of vaginal fluid has such a configuration. In preparations of vaginal fluid there is a significant correlation between the occurrence of spindle-shaped cells and the presence of induced cancer in the cervix of the mouse.

The observation made by von Haam regarding withdrawal studies is of considerable interest. Those mice having spindle-shaped cells in their vaginal fluid before the carcinogen was withdrawn were all ultimately proved to have cancer. Our own experience would indicate that these animals already had cancer at the time the carcinogen was withdrawn.

I would disagree with von Haam only in regard to the site of origin of the spindle-shaped cells. In my opinion, these cells reflect an abnormal keratinization in the parent tissues. Because of this the cells are more abundant in the presence of keratin forming cancers. In the human this is characteristic of keratinizing cancers of the urinary bladder, lung, oral cavity, skin, and uterine cervix. Such cells are less common or non-existent in the presence of large cell non-keratinizing cancers[4]. With the thread technique in the mouse almost all the resultant cancers are either of the keratinizing type or have a keratinizing component.

The more elongated spindle-shaped cells observed in the presence of induced cancer are often observed to have a delicate linear fibril extending the length of the cell. This in all probability is analogous to the tonofibril which is currently believed to be the precursor of keratin formation in skin. This fibril might also explain the unusual configuration of the cell[3].

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CLOSING REMARKS

EMMERICH VON HAAM, Columbus, Ohio, U.S.A.:

I am gratified that Dr. Reagan's experiences with experimental carcinoma of the cervix of mice are in such close agreement with our own findings. We have now adopted for our future experiments the method of Murphy and find it much better than our previously used method of painting the cervix with the carcinogen since it is technically more simple and also avoids unnecessary traumatization of the tissues. I do not think that the spindle or fiber shape of those cells has anything to do with the actual process of keratinization since some of the cells remain quite basophilic, and we have not observed characteristic keratohyalin granules in their protoplasm. I do agree that spindle-shaped cells are practically non-existent in the highly undifferentiated or small cell cancers which we produced in our animals. We are now repeating our withdrawal experiment on a large scale and are paying close attention to the changes of the cytological picture after carcinogen withdrawal. By utilizing the string method for these experiments, we feel more certain of removing most of the carcinogenic material at a given time period than we were in our previous experiments.

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COLPOMICROSCOPY ON SPINDLE-SHAPED SQUAMOID CELLS

T. ANTOINE, K. BRANDL, V. GRÜNBERGER, E. KOFLER, AND H. KREMER
Wien, Austria

One can observe fiber cells in the colpomicroscope. They correspond with the cells found in cytology and histology. The nucleus of the fiber cells as seen in the colpomicroscope is spindle-shaped, cigar-like, and hyperchromatic with a sharp nuclear border. Nuclei are more likely to be found in clusters than in a single line, and they are sometimes concentrically arranged. According to cytology the fiber cells are generally noted in squamous cell carcinomas that are already invasive.

The following types of cells have to be differentiated from the so-called fiber cells:

1. Rolled-up normal superficial cells which are mainly formed artificially in the course of examination by the friction of the tube attached to the surface of the cervix. Similar confusion can arise by means of folding of the cytoplasm of the cells through pressure of the tube of the colpomiscroscope on the surface of the cervix.

2. Furthermore, in the area of a true erosion (erosio vera) some young connective tissue cells are to be noticed.

3. In the course of the healing process of an erosion, especially on its margin, concentrically located squamous cells can be seen. These may be somewhat similar to fiber cells.

All these benign cell types differ from the true squamoid fiber cells by their size and, above all, by their lack of nuclear chromatin abnormalities.

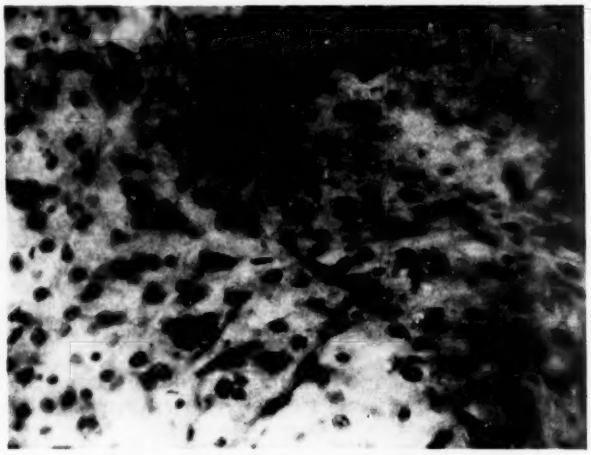

Fig. 1. Invasive squamous cell carcinoma of the cervix.

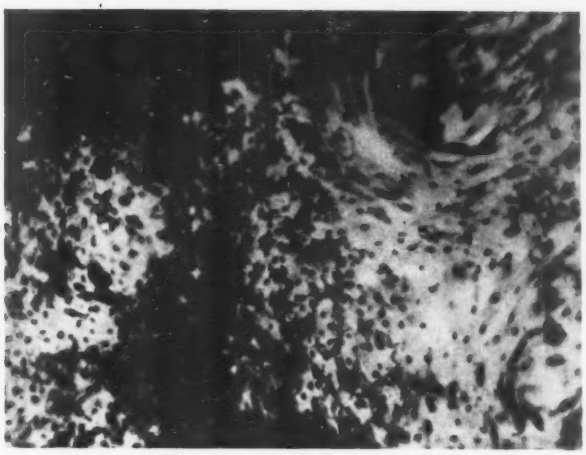

Fig. 2. Glandular erosion undergoing epidermization.

WOLFGANG WALZ
Heidenheim a.d. Brenz, Germany

Spindle cells are seen in colpomicroscopy. As in cytological examination they are recognized by their elongated cytoplasm and their lengthy and often intensively colored nucleus. Additional details cannot be seen under the colpomicroscope as we normally stain with toluidine blue and the upper limit of enlargement exceeds factor 200. For cytology, however, it is of importance to know in which lesions such spindle cells appear and where they are localized.

In epidermization zones or in inflamed epithelium, spindle-shaped cells can occasionally be found surrounding the orifice of a cervical gland duct and connective-tissue papilla, reaching close to the squamous epithelial surface. They probably correspond to cells which are found occasionally in the course of cytological examination of such areas.

Spindle cells are found in colpomicroscopy in increasing numbers on the surface of dysplasias, still more so in carcinoma in situ and invasive carcinoma of the cervix. When they are more differentiated they are much more frequently found, while they are found to a lesser extent in carcinomas showing less differentiation. This observation is well known in cytology as well.

As far as localization of spindle cells in carcinomas is concerned, it was noticed that they usually surround the connective-tissue papillae (Fig. 1). They cannot appear anywhere else on the surface of such lesions, however. It is remarkable that they very rarely appear singly. Usually a number of spindle cells line up one after another (Fig. 2).

Especially when spindle cells surround connective-tissue papillae, we would like to draw the tentative conclusion that such spindle cells as types probably resemble less differentiated cells. Where the connective-tissue papillae come to the surface, cells of lower layers of the epidermis reach to the surface. Here they adapt themselves to surface conditions by trying to assimilate their shape under new conditions.

Fig. 1. Surface of invasive squamous carcinoma of cervix. Many connective-tissue papillae reaching the surface (intensively stained) surrounded by spindle cells (as indicated). Stained by Toluidin blue. Enlargement factor 180. Under colpomiscroscope.

Fig. 2. Surface of a carcinoma in situ under colpomicroscope. Chain of spindle cells (as indicated). Stained by Toluidin blue. Enlargement factor 180.

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DISCUSSION

JOHN F. SHEEHAN, Omaha, Nebraska, U. S. A.:

Colpomicroscopy is a method of surface microscopy for direct in vivo studies of the supravitally stained epithelial surface of the cervix (1, 2). Normal, atypical and malignant squamoid cells of the portio viewed under colpomicroscopy reveal morphologic detail equivalent to similar cell types found on cytological smears. Colpomicroscopy provides a method for direct visualization of hyperchromasia, anisokaryosis, and anisocytosis in undisturbed sheets of superficial cells of the portio, stained supravitally by hematoxylin, toluidine blue or Evans' blue, according to the method of Antoine (1), Grünberger (3), Walz (4), and others. We are in complete agreement with the authors that the precise morphologic criteria under colpomicroscopy for recognition of elongated malignant fiber cells is the appearance, size and shape of the nucleus. Malignant fiber cells reveal elliptical, intensely hyperchromatic, dense nuclei similar to the nuclei of the classical malignant squamoid fiber cells of cytological smears.

In our limited series examined to date by colpomicroscopy, fiber cells have been observed in cases of proven differentiated epidermoid invasive cervical carcinomas. Superficial necrosis may interfere with visualization of cellular detail. Usually the fiber cells appear in whorls or in strata (Fig. 1, 2) as the authors mention. Benign fiber-shaped cells may show pyknotic nuclei or palely stained granular nuclei in contrast to the densely stained nuclei of malignant squamoid fiber cells. Cytological smears prepared directly from a scraping of the supravitally stained portio, fixed in ether-alcohol and counter-stained with EA 36 indicate that the nuclei of benign and malignant cells as visualized under the colpomicroscope are similar in appearance to nuclear types found on cytological smears.

A distinct advantage of colpomicroscopy is the potential usefulness for studying, by repeated examinations over a long period of time, in situ cytology of the cervix. Colpomicroscopy, as an adjunct to cytology and biopsy, has potential value in detecting early cancer of the cervix, whereas other methods might occasionally fail (5). As stated by Antoine (1), "The different methods are not rivals and cannot substitute for each other, but the more methods used the better the results will be."

The opinion of the authors is invited concerning the desirability of dropping the term "fiber cell" and substituting the term "snake cell," as suggested by Papanicolaou (6). The term "fiber cell" gives the impression that these cells are derived from connective tissue.

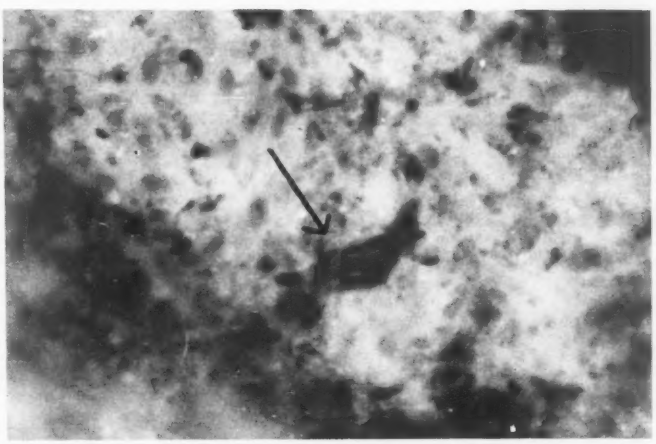

Fig. 1. Malignant squamoid fiber cells in area of invasive differentiated epidermoid carcinoma of cervix. Under colpomicroscope. Stained by Harris hematoxylin. 210X.

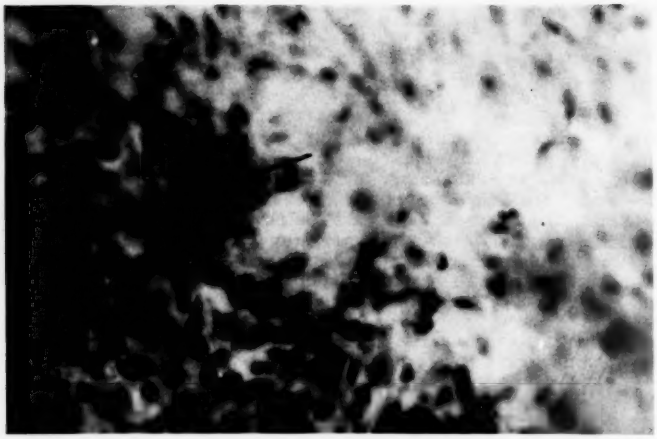

Fig. 2. Spindle-shaped nuclei of malignant squamoid fiber cells. Under colpomicroscope. Stained by Harris hematoxylin. 210X.

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CLOSING REMARKS

T. ANTOINE, Vienna, Austria:

We appreciate the details discussed on this subject and are in full agreement. We also fully agree with the substitution of the term fiber cell for the term snake cell suggested by Papanicolaou.

WOLFGANG WALZ, Heidenheim a.d. Brenz, Germany:

No comments.

OCCURRENCE OF SPINDLE-SHAPED SQUAMOID CELLS IN INFECTIONS

GUILLERMO TERZANO
Buenos Aires, Argentina

During the acute phase of infections, when there is purulent discharge and the vaginal fluid contains leukocytes, red blood cells, degenerated cells, cellular debris, etc., it is easy (when dealing with smears) to overlook the presence of spindle-shaped cells or to interpret them erroneously.

When the acute infection has ceased, but the cervical or vaginal mucosa remains congested, showing edema, redness, sometimes friability, etc., abnormal cells (including bizarre forms) may be present in smears.

Spindle-shaped cells (isolated, in groups or in pearl-like formations) are seen in smears of women having infections (gonococcus, streptococcus, staphylococcus and other pyogenic organisms). In a study of the vaginal cytology in a random group, spindle-shaped cells were found in 5 (2.2%) of 220 women having infection and in 6 (1.9%) of 310 routine vaginal smears of women without any evidence of infection. In chronic infections, such as tuberculosis, spindle cells have been seen in the smears of two women, both having tuberculous endometritis with an apparently healthy cervix.

The hormonal status of infected women does not seem to have a special role with the presence of these cells in smears. Of the 5 mentioned cases, 2 were post-menopausal, 3, normally menstruating women. We have never seen spindle-shaped cells in smears of young girls before menarche; the group was studied because of vulvitis.

The nucleus (one or two) of the spindle cells found in infections may sometimes show a little hyperactivity. When there is no other type of abnormal cells present, the report does not go beyond Papanicolaou's Class II. The cells are identified as benign, based on the benign appearance of the nucleus, no matter how bizarre they may look (spindle, fiber, snake and even filiform type).

We do not consider ourselves qualified to make a special statement based only on the presence of spindle-shaped cells in smears, because we have not been able to find a real relationship between the presence of those cells and infection. Also, according to the definition given by the Editorial Office, I would like to emphasize the fact that in smears of women having infection the so-called pseudo-spindle cells, the degenerated cells and the rolled-up cells are found more frequently than the actual spindle-shaped cells. (Unfortunately we did not keep a record of the frequency of the pseudo-spindle cells.)

DISCUSSION

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

I agree completely with Dr. Terzano when he describes as follows the spindle cells which occur in infections:

Cytoplasm: bizarre-looking (spindle-shaped or even filiform).
Nucleus: appears benign, even if sometimes exhibiting a hyperactive appearance.

I would designate these cells rather as "pseudo-spindle cells." In material from approximately 49,000 women, I have never seen "real spindle cells" with malignant nuclei in patients with inflammation. Rather, I have seen "real spindle cells" only in atypical epithelial proliferation (carcinoma in situ, invasive squamous carcinoma or dysplasia).

I would like to ask Dr. Terzano whether he can give, in his cases, the frequency figure in vaginal, ecto- and endocervical smears. In my cases pseudo-spindle cells are found almost exclusively in scrapings from the cervix surface and in the endocervical smear, but almost never in the vaginal smear. I maintain, therefore, that it is possible that these cells are not an expression of vaginitis, but are frequently associated with erosion and erosion healing and so belong in the group of regenerative epithelium (respectively, metaplasia).

From the foregoing it becomes clear that:

1. pseudo-spindle cells do not appear in all infections.
2. their appearance is independent of the hormonal situation.

JULIETA C. de LAGUNA, Mexico, D. F., Mexico:

The points raised by Terzano are very interesting. Indeed, non-malignant spindle-shaped cells are very seldom observed. We agree that smears with those cells are, at most, Negative II. Pearl-like formations carry no significance of malignancy whatsoever. (I wonder if all the cells that form these pearls may really be considered as spindle-shaped.)

It would be convenient to establish the difference between "pseudo spindle" (I agree with Terzano that they are very common), "spindle-like" (those seen in benign conditions) and actual "spindle-shaped" cells (malignant).

CLOSING REMARKS

GUILLERMO TERZANO, Buenos Aires, Argentina:

It is our understanding that to correctly classify a spindlé-shaped cell as benign or malignant, the cytologist depends primarily on the morphological aspect of the nucleus of the cell. The shape of the cell, no matter how bizarre it is, will not mislead him to give a positive diagnosis if the nucleus does not show malignant features.

As Julieta de Laguna says, when pearl-like formations are present in the smear and spindle-shaped cells are seen among the components of the pearl, it is also the abnormal nucleus in this case that will lead to a decision between a positive or negative diagnosis.

In the 11 cases in which spindle-shaped cells have been observed, seven were specimens from cervical scrapings and four were from vaginal aspirations. This does not mean that the cells were derived from the vaginal epithelium. In our series, all the cells were of the squamous epithelial type (never of the glandular type), looking more like cells of ectocervical origin than of vaginal origin.

I am glad that Boschann brought up such a good point. It is also our feeling that this type of cell is an expression of erosion and erosion healing. This opinion seems to be strengthened by the fact that spindle-shaped cells, tadpole cells, etc. (Class II and Class III) are particularly numerous in smears of patients in whom diathermo-coagulation of the cervix was performed, and also in cases where there are granulation tissues.

It is gratifying indeed that such good observers as Boschann and Julieta de Laguna agree that actual spindle-shaped cells are not as frequent in infections as arethe pseudo-spindle cells, the tadpole cells, etc.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

OCCURRENCE OF SPINDLE-SHAPED SQUAMOID CELLS IN DYSPLASIA

JEAN de BRUX, JACQUELINE DUPRÉ-FROMENT and ANDRÉ RAUZY
Paris, France

Spindle-shaped squamoid cells arise from an epithelial lesion which may be benign, suspicious or malignant. When considered alone, these cells cannot be used to give a definitive diagnosis. However, their presence alerts the cytologist and makes him examine the material more closely for other abnormal features.

Morphology:

The appearance of fiber cells is very characteristic: they have an elongated, spindle-shaped form with regular borders. Their cytoplasm is broad at the level of the nucleus and tapered at each end. Sometimes they are drawn out like a ribbon, with indistinct borders; at other times they seem to be in a transitional form resembling tadpole cells. Their size is variable, but usually the widest diameter is greater than that of a parabasal cell. Fiber cells can occur singly or in clumps, or be arranged in chains or whirls, forming "parakeratotic epithelial pearls."

Their cytoplasm is usually homogeneous without inclusions or vacuoles. In pregnancy it may be foamy; generally it is eosinophilic. The nucleus is large, regular and vesicular. It is always centrally located with its edges in contact with the cell membrane, and of variable shape: round, oval or elongated. In other cases folding of the chromatin gives it a budding appearance. If it is pyknotic, without perceptible structural detail, it appears abnormal due to its great length.

Etiology:

There are two types of dysplasias in which fiber cells occur:

(a) those in which the epithelium is modified by general trophic influences. These occur in hypo- and hyper-hormonal conditions and in pregnancy.

(b) those in which a local factor of an inflammatory or parasitic nature is acting.

In (a), fiber cells may appear during the menopause, but they are infrequent and may be passed over. Their presence in these cases is almost always associated with an inflammatory appearance which seems to be the causative factor, rather than the hypotrophism.

We have never seen fiber cells in hyper-estrogenic smears with no concomitant lesion. In fact, one is struck by the cleanness of the smear and by its uniformity, consisting exclusively of superficial cells which lie singly and flat, brightly staining and completely eosinophilic, with a pyknotic or pre-pyknotic nucleus, or even anucleate squames.

In pregnancy, a complex hormonal state in which apart from estrogens and progestogens, chorionic gonadotrophins are acting, the well-known histological modifications of the cervix are reflected in smears as a simple dyskaryosis affecting basal and intermediate cells.

The nucleus is large, sometimes even budding, but with chromatin that is finely articulated and regular. On the other hand, where there is a super-imposed cervical lesion, one can find fiber cells, with a cytoplasm that is often finely vacuolated, blue-gray or polychromatic, and a nucleus that is large and has a pyknotic tendency.

To summarize, it seems permissible to say that in all cases where changes are due purely to functional and non-specific causes, fiber cells are not encountered; they are seen only when the lesion is of organic origin, i. e., due to a direct local action which causes a dystrophia affecting a localized area of the cervix.

In (b), we are dealing with cases of inflammation and infestations; the fiber cells are numerous and are surrounded by other cells equally dysplastic, with pyknotic nuclei, with a premature eosinophilia of the cytoplasm and perinuclear halos; the latter sign in particular is characteristic of Trichomonas infestation. The smear is "dirty," full of leukocytes and histiocytes.

In leukoplakic dysplasia, the fiber cell constitutes one of the most significant cellular elements, side by side with anucleate squames and free keratin. Fiber cells are, in these cases, always eosinophilic and sometimes orangeophilic with eleidin granules. The nucleus varies in appearance, being either clear and vesicular or darker with a tendency to pyknosis. Finally, in cases of metaplasia from ectropion or ectopy, fiber cells showing the same morphology may be seen, although less frequently.

Cytogenesis:

Having obtained the above clinico-anatomical data, one can postulate that fiber cells are cells that have reached the intermediate stage, are normal as judged by their detailed structure, but may be modified by various factors such as inflammations, toxins, changes of pH, enzymatic and hormonal influences (and other factors of which we are ignorant). In one respect, the fiber cell could be the end result of degenerative changes acting on cellular elements already differentiated but not yet having reached complete maturation.

The two constituents of the cell will be attained as follows:

(a) The cytoplasm becomes eosinophilic or orangeophilic as in the background of parabasal cells, and undergoes a change which gives the cell its characteristic elongated form, as one also sees in different stages of degeneration from post-irradiation smears.

The "glycogen and mucous secreting" character of the cytoplasm disappears. The reaction of proteins bound to sulph-hydril and disulphide radicals appears prematurely, which demonstrates histochemically that these cells mature rapidly and prematurely.

(b) The nucleus, having undergone a fairly rapid retraction, appears either in a budding and irregular form, due to folding of the chromatin, or in a partial pyknosis. Finally, sometimes the nucleus is little affected and remains clear, vesicular and even swollen.

As we see, the fiber cell sometimes appears in dysplasias. This is evidence of abnormal maturation of the cell, the abnormality not affecting the deeper germinal layers. Chemical factors, cytological and pathologico-anatomical findings and their mode of development all serve to prove this; these factors will be discussed from another viewpoint in the paper on spindle-shaped squamoid cells in carcinoma in situ.

MANUEL GALBIS
Valencia, Spain

The term "dysplasia" represents a somewhat vague concept used to designate the structural changes, other than those of carcinoma, of the stratified squamous epithelium of the cervix. In order to classify these pathological changes of the epithelium we prefer to use the nomenclature proposed by Glatthaar, who differentiates the physiological changes, such as ectopia, regenerating epithelium and metaplasia, from those definitely pathological entities with changes in the cellular architecture and structure, calling these "abnormal epithelium" (in the sense given by Glatthaar), "epithelial disorder" (unruhiges epithel) and "atypical epithelium."

We are not going to discuss the significance of such epithelial abnormalities or their relationships to carcinoma. In our classification we have excluded any neoplastic changes, whether invasive or not. Accordingly, we are not considering as dysplasia intra-epithelial carcinoma, which has a definite histological pattern.

In the Clinic of the University of Valencia Medical School, cancer detection is accomplished by the screening of material previously selected and by the routine use of cytology and colposcopy; further confirmation is by biopsy. Our experience has demonstrated the well-known conclusion that there is not a strict relationship between the colposcopic cytological findings and the histological picture, as far as dysplasias are concerned. We have drawn the following conclusions concerning the presence of spindle cells in the smears:

1. In every case in which colposcopy showed an epithelium diagnosed as benign (original epithelium, erosio vera, ectopia, transformation zone), the pathological study failed to reveal a picture of dysplasia. The spindle cells were found very rarely or not at all. Accordingly, the presence of spindle cells in a smear reveals, in nearly every case, the existence of a pathological epithelium, neoplastic or not.

2. Spindle cells are found practically only in smears corresponding to atypical colposcopic pictures, such as leukoplakia, "base" (grund), "felderung" and atypias. The corresponding histological picture of these cases varies from abnormal epithelium to carcinoma in situ or invasive carcinoma.

Of all the cervical smears studied in which spindle cells were found, only 13% of the cases correspond to epithelial dysplasias. The remainder were cases of carcinoma, invasive or non-invasive.

We have not found a strict correlation between the degrees of epithelial abnormality and the frequency of spindle cells in the smear. These can be found in the same numbers of an "abnormal epithelium" (in the sense given by Glatthaar), as in epithelial disorders, or atypical epithelium. Their presence seems to depend more upon the degree of cellular maturation and parakeratosis.

We have found, therefore, that spindle cells may appear in the smears of any of the non-neoplastic epithelial abnormalities. Their frequency is much higher in carcinoma, but does not warrant the cytologic diagnosis of neoplasia unless definite nuclear changes are found.

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

Over a period of years we were able to collect twenty-four cases of atypical epithelium with positive vaginal smears. A number of the cases had been called carcinoma in situ originally, but on review a more conservative opinion was given and they were reclassified as "atypical epithelium." The vaginal smears from these patients all contained cells which we considered to be malignant. However, in only two cases or eight per cent of the twenty-four were there fiber or spindle cells. From this evidence we conclude that fiber or spindle cells are rarely a component of the cellular picture in cases diagnosed histologically as having atypiçal epithelium.

DISCUSSION

JEAN BERGER, Basel, Switzerland:

To the question of occurrence of fiber cells in the different modifications of squamous epithelium of the cervix, we can, in agreement with Galbis, Graham and de Brux, say that those cells can be seen in the benign as well as in the malignant modifications. Thus, fiber cells have no specificity. In the smears from normal epithelium we very seldom see them. We can find them only when inflammations or modifications engendered by irradiation have changed the normal cells into spindle-shaped, stretched cells--corresponding to the fiber cells.

In dysplasia or marked atypical epithelium (Hinselmann III/IV a, b, c,) spindle cells occur with greater frequency the more differentiated and mature the epithelium is. Here we recognize most stretched cells with eosinophilic cytoplasm and hyper-chromatic, elongated to longitudinal-oval nuclei.

We classify smears in which fiber cells can be found (with eosinophilic or basophilic cytoplasm) into Group III (suspect: Papanicolaou). In these cases, a repeat smear and a histological verification are always necessary, whereby, in more than 40% a dysplasia or a marked atypical epithelium, if not a beginning invasive carcinoma, is to be expected.

LUIS MONTALVO-RUIZ, Madrid, Spain:

In agreement with de Brux and co-workers, we believe that spindle cells, appearing as often in benign as in malignant lesions, do not permit us to make a definite diagnosis. We have found them in normal smears, in dysplasias and in carcinoma.

Spindle cells are found with greatest frequency in the Class III smears (Papanicolaou), associated with dyskaryotic and tadpole cells, and are confirmed histologically to be from an atypical epithelium.

We encounter them in regular proportion in carcinomas. We also observe their presence with relative frequency in inflammatory processes. Finally, in Class I cases without histological epithelial lesions, we very rarely have found spindle cells.

In view of the above, we believe that although the presence of spindle cells is more frequent in dysplasias, they are not exclusively from this type of lesion and are degnerated cells. This means that when we see spindle cells in the smears, we follow the patient by a more complete exploration with colposcopy and selective biopsy.

VIOLETTE NUOVO, Paris, France:

From October, 1949, to December, 1957, among the 32, 333 patients who had vaginal and cervical smears in our cytology laboratory, 162 patients had a pathological report of dysplasia (0. 5%). The cytology reports were as follows: 64: Class II; 76: Class III; 22: Class IV.

Among these 162 patients, only eight presented fiber cells on the smears. This represents a very low percentage: 4%. These patients had the following cytology reports: none: Class II; 6: Class III; 2: Class IV.

Besides, the term "dysplasia" is very vague. It includes too many lesions from the very benign ones to the highly suspicious ones, and the borderline between these and cancer in situ is sometimes very difficult to determine. Along with Dr. Graham, we will conclude from this evidence that fiber cells are rarely seen in the vaginal and cervical smears of cases diagnosed pathologically as having cervical dysplasias.

HORST SMOLKA, Kiel, Germany:

From the three main papers we see how difficult it is to define the term "dysplasia" exactly and how many possibilities of origin may have to be reckoned with. It seems that R. Graham and Galbis have found spindle cells in cases of the same kind, i. e. particularly in cases of atypical epithelium. This corresponds to our observations. Furthermore, however, we have also occasionally seen spindle cells in metaplasias of the endocervix presenting a pathological cell pattern. In these cases we found marked fiber cells associated with other cells of the dysplastic parabasal types, such as spider cells and tadpole cells. This shows that fiber cells or spindle cells may originate from epithelia of less differentiation, too, though when they stain eosinophilic with pyknotic nuclei, we also think of them as characteristic of a high maturity of epithelia, especially parakeratotic epithelial pearls. Particularly de Brux and his collaborators have pointed this out already.

I am glad that Graham and Galbis have stressed that spindle or fiber cells occur very rarely in non-malignant tissues. I too can fully emphasize this fact. However, I am astonished that de Brux and colleagues also observe fiber cells in great numbers in inflammations.

Single spindle cells may occasionally be found associated with only few other pathological cells in nearly normal smears. According to our experience it is often not possible to determine their origin if there are no clinical symptoms, and also after thorough clinical examination including biopsy. We have, therefore, come to the conclusion that in these cases extremely small dysplastic regions of epithelium (for instance, metaplasias) are likely to be the places of origin.

At any rate, we wish to emphasize that in many cases it is scarcely possible to determine whether or not spindle cells occurring singly in a smear originate from a carcinoma. As the shape of the cell is always atypical, only the judgement of the nucleus could lead to a result. The nuclei are frequently so dark, however, that one cannot decide whether it is a harmless pyknosis, merely a sign of marked maturity or a pathologic hyperchromasia.

For these reasons, I would like to point out that when spindle cells are found, it is very important to search very carefully for further pathological signs in the smear in order to exclude a carcinoma.

CLOSING REMARKS

JEAN de BRUX, Paris, France:

See my closing remarks to the discussion of the paper entitled "The Occurrence of Spindle-shaped Squamoid Cells in Carcinoma in Situ" in this edition.

MANUEL GALBIS, Valencia, Spain:

In summary, there is general agreement of the discussants on the relative rarity and specificity of spindle cells in dysplasias. They can also be present in malignant lesions, but with greater frequency than in dysplasias. The presence of spindle cells in a normal epithelium is very rare; Smolka observes that in these cases there are probably small areas of dysplasia. The fact that they can be found occasionally in endocervical metaplasia is proof that they have originated from a less differentiated epithelium.

The discussants are in agreement in considering the presence of spindle cells in the smears as suspicious. Nuovo only finds them in the smears of type III or IV; Montalvo in those of type III; Berger classifies the slides with each cell as type III; and Smolka insists on the importance of looking carefully for carcinoma in such smears. I am completely in agreement with these opinions; the presence of spindle cells causes us to consider the smears as suspicious and, therefore, it is necessary to carefully investigate the case. If spindle cells are observed in a dysplasia, there are very few, less in number than in a carcinoma. We have never seen a case of dysplasia with abundant spindle cells in the smear.

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

In these discussions by Nuovo and Montalvo two completely divergent views are presented. One, that fiber cells occur only rarely in dysplasia, the other that spindle cells are more frequent in dysplasia. Are we all talking about the same cells? I think not. Montalvo writes "We also observe their presence with relative frequency in inflammatory processes." I understand from this statement that these are inflammatory processes without associated malignancy. If this is true then Montalvo is not considering the same cells that Nuovo and I are discussing. The fiber cell, in my opinion, is a malignant cell cytologically. It is the configuration of the chromatin in the nucleus which stamps a cell as malignant, not the shape of the cell.

Berger appears to have misunderstood my discussion since I did not say that fiber cells can occur in benign conditions. I do not consider dysplasia as a benign condition. As I pointed out, the majority of the cases now classified as dysplasia, in the cases that I reported, were originally called carcinoma in situ, indicating that the epithelia were so abnormal that some observers thought they should be classified as carcinoma in situ. Such epithelia can hardly be called benign.

I agree with Smolka that the finding of fiber cells in great numbers in inflammation not only is astonishing but confusing. We cannot be speaking of the same cell. That elongated cells occur in the presence of inflammation I have no doubt, but these cells still have a smooth chromatin pattern.

Smolka makes an interesting point in his discussion of single fiber cells. When one considers a single fiber cell, it is sometimes difficult to be sure the cell is malignant because of the extreme pyknosis obscuring any nuclear detail. However, from the practical standpoint, for such cells to occur alone, without either fiber cells in which the chromatin irregularity can be seen or unaccompanied by other distinctive malignant cells, is extremely rare.

I have the distinct impression from these discussions that two different cellular entities are being considered. One is a benign cell with a regular nuclear pattern and an elongated shape. Its deviation from the normal is not in the nuclear pattern but in the configuration of the cytoplasm. The second cell under discussion is also elongated in shape, but in addition has either a large elongated pyknotic nucleus or a nucleus with distinct chromatin aberration. I feel that these are two distinct cells which can be identified separately and should not be confused. Confusion will exist as long as both are described under a single heading of fiber or spindle cell.

OCCURRENCE OF SPINDLE-SHAPED SQUAMOID CELLS IN CARCINOMA IN SITU

JEAN de BRUX, ANDRÉ RAUZY and JACQUELINE DUPRÉ-FROMENT
Paris, France

The fiber cell is one of the elements of the characteristic complex of cells seen in "regular dysplasias" as well as in carcinoma in situ.

Although theoretically it has different potentialities in the two conditions, there is no means of judging by its appearance whether it is benign or malignant.

Let us consider one by one the following facts: Fiber cells in "regular dysplasias" and in carcinoma in situ have an almost identical appearance. Their cytoplasmic features, shape and staining reaction are the same as in all fiber cells. One may see a cytoplasm that stains red or orange, is very fine and elongated, or an obvious cyanophilia in a short, thick cell.

The nucleus is very characteristic: it is more or less oval in shape with a very regular outline, very dark, showing a uniform mass of chromatin; the nuclear-cytoplasmic ratio may be reversed, a fact in favor of probable malignancy, but one which is rarely seen.

In fact, in most instances one sees clumping of the chromatin with notching of the nuclear membrane, giving the appearance of a deeply staining nucleus that is large and irregular, but which appears to be in the process of retraction.

Fiber cells are only one element of a cytological picture which can give a probable, if not certain, diagnosis. In fact, the cells seen in association with fiber cells are as follows:

1) similar cells, relatively differentiated and mature, e.g., tadpole cells.

2) intermediate and superficial cell types with eosinophilic cytoplasm and a nucleus that is of atypical shape, with a thickened cell membrane and clumping of the chromatin.

3) immature basal cells which have an abnormal nucleus, the surrounding cytoplasm having an irregular edge, with cyanophilic staining reaction, sometimes vacuolated or in the process of degeneration.

It is by studying the cellular picture of the environment that one can get some idea of the lesion from which fiber cells are exfoliated. Similarly, pathological anatomy gives one the architectural pattern of the lesion and helps one to understand the cellular elements seen in smears.

The histological pattern of carcinoma in situ shows a multi-stratification of the elements, arranged perpendicularly to the basement membrane, without differentiation of the cells into regular layers. But on the surface of the epithelium one can discern an attempt at differentiation. The cells become rounded and appear like those cells which in smears we call "parabasal," with either cyanophilic or orangeophilic cytoplasm but a large nucleus with an early condensation that gives it a dense and intensely staining appearance.

It is only on the very surface of the epithelium that the true fiber cell appears, with its characteristic thin, elongated form. These observations will be associated cytologically with a high percentage of immature cells, with respect to fiber cells and, in general, with respect to eosinophilic elements.

In "irregular dysplasias," differentiation occurs at an earlier stage. The intermediate cell layer appears in its usual place but with certain changes such as the absence of intercellular bridges, the persistence of large nuclei having a dense chromatin, and an intensely eosinophilic cytoplasm. In vaginal smears one finds a predominance of mature elements as judged from the cytoplasmic viewpoint, but relatively immature as regards the nucleus. One is guided more by the frequent occurrence of eosinophilia than by nuclear changes, which in this case are not particularly marked.

The difficulty facing the pathologist as well as the cytologist is to discriminate between a reversible, dysplastic lesion or one which at least will remain stationary, and a lesion which is an actual carcinoma in situ, and therefore one that will inevitably become invasive. The number of hysterectomies performed in the name of "carcinoma in situ" is such that we do not make this diagnosis except with very strict criteria. The best method of approach, in our opinion, is to compare the pathologic lesion with the cytological smear.

During the study of several cancers considered in biopsies to be intra-epithelial but which in the operation specimen subsequently proved to be invasive in one area, we were able to establish a criterion for differentiating mature from immature elements. If the number of abnormal immature elements in a smear is greater than 30%, the lesion is probably a cancer. Thus, by bringing the pathological and cytological findings side by side one can get some idea of the genesis of spindle cells in carcinoma in situ and in "irregular dysplasias." We are dealing with a cell which, in both these lesions, appears at first morphologically the same: a basal cell which is fundamentally abnormal (to a variable extent) derived from another abnormal cell. It gradually reaches the surface of the epithelium but does this by different methods in the two lesions.

In carcinoma in situ, from the point of view of architectural pattern, the property of differentiation has practically disappeared (it is understood that these statements do not apply to invasive cancer).

In "irregular dysplasia," although it is equally disorderly in appearance, there is, on the contrary, a tendency to differentiation; as we already know this is still very abnormal.

All these pathogenetic considerations, explained by comparative studies, should not make us lose sight of the practical advantage of looking for fiber cells in some smears. The cytological picture which we have described and for which we have given criteria for interpretation should always be studied, not purely for its morphological abnormalities, but in an attempt to decide from which type of lesion the cells are exfoliated.

SUMMARY

1) The fiber cell has no specificity of its own. It can be seen in benign lesions as well as in suspect or malignant lesions. In the latter, the cell presents malignant features as seen in any other cell type.

2) It always arises from an organic epithelial lesion and does not appear in any other circumstance.

3) In "regular dysplasias," its detailed structure is normal, but modified during growth by various local factors. In "irregular dysplasias" and in carcinoma in situ it is abnormal from the start and always shows abnormal features.

4) It is an abnormal cell but a mature one. Its presence in a lesion is a sign of differentiation, very marked in "irregular dysplasia" but much less so in carcinoma in situ.

One can say, therefore, in summary that the fiber cell on its own has no significance: it depends entirely on the concomitant features, and these alone permit one to make a definite diagnosis, taking into account the degree of differentiation of the cellular elements, for one can be quite certain that the more fiber cells there are present, the greater chance there is of the lesion being benign.

MANUEL GÀLBIS

Valencia, Spain

Spindle cells are seldom found in smears corresponding to normal epithelium. They are occasionally found in non-neoplastic, abnormal and atypical epithelia. The majority of smears in which spindle cells are found correspond to well-differentiated carcinoma. In such cases the nuclear changes are often very significant.

Carcinoma in situ is not a dysplasia but a true carcinoma. Its histological characteristics are those of a neoplastic epithelium, without invasion of the connective tissue. The concept of a carcinoma in situ is histological, never cytological. The cytologist can make a diagnosis of carcinoma only from the presence of malignant cells in the smear, and even then he may make a mistake in diagnosis.

In this sense, a carcinoma in situ may show spindle cells with malignant characteristics, the same as an invasive carcinoma. The presence or absence of spindle cells in the smear does not give information as to the invasiveness of the tumor, but only its cellular differentiation. We rarely make a diagnosis of carcinoma in situ; after a careful and meticulous study we found that a number of cases turned out to be just areas of atypical epithelium; in other cases further biopsies demonstrated the presence of an invasive carcinoma.

In our few cases of carcinoma in situ, we have found spindle cells in only 18%. We believe that the presence of spindle cells is not a significant feature of a carcinoma in situ.

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

As in the case of atypical epithelium, the surface cells of carcinoma in situ are not usually fiber cells. Fiber or spindle cells are seen only infrequently in the exfoliated cancer cells of carcinoma in situ. In reviewing the type of malignant cell present in 56 cases of histologically proven carcinoma in situ, in only one instance were fiber cells encountered in a differential count of 100 cancer cells.

DISCUSSION

FRITZ BAJARDI, Graz, Austria:

Any inference to be drawn from the presence of spindle cells in a smear of surface carcinoma must largely depend on the histological diagnosis, about which there is still considerable difference of opinion. The symposium dealing with the histology of surface carcinoma, which will be published in this journal, may lead to closer agreement on this subject.

In my own opinion, carcinomas in situ are nearly always undifferentiated and the occurrence of spindle cells in such cases is exceptional. On the other hand, in those rare cases of mature surface carcinomas, spindle cells will be found in the smears.

JEAN BERGER, Basel, Switzerland:

Since we find "fiber cells" in smears of carcinoma in situ as well as in smears of early invasive carcinoma, these cell types give no criteria of a definite diagnosis. Here, only histology can decide if it is an invasive carcinoma or not. The appearance of fiber cells permits only the conclusion that a differentiation and cell maturation in the tissue exists or does not exist. (See also A #10 discussion.)

HANNS-WERNER BOSCHANN, West Berlin, Germany:

To Dr. Galbis:

In our material from over 47,000 women, spindle-shaped cells differed considerably in non-malignant and malignant changes.

In non-malignant changes we see "pseudo-spindle cells." They differ in malignant changes from the real spindle cells by the fact that their nuclei in morphology and cyto-chemistry correspond to the nuclei of normal cells with corresponding states of maturity (and thus show fine granular chromatin and a round regular membrane). The nuclear-cytoplasmic ratio is not displaced in favor of the nucleus, while in its form the cytoplasm can slightly resemble the form of spindle-shaped cancer cells. Such "pseudo-spindle cells" are a frequent occurrence in erosion healing.

In malignant changes we see real spindle cells but only in squamous epithelial carcinoma. For practical cytologic diagnoses, their pathognomonic oocurrence provides assurance in diagnosing an irreversible lesion of the squamous epithelium and in excluding an adenocarcinoma. In

comparative evaluation of 73 carcinomas in situ and 73 invasive carcinomas, we find spindle-shaped cells with somewhat similar frequency in both types of carcinoma (without prekeratin formation, 25:20; with prekeratin formation, 25:31). Therefore, I agree with the opinion of Dr. Galbis that the presence of spindle cells is not a significant feature of a carcinoma in situ (cf. my opinion in the discussion of "Are spindle-shaped squamoid cells suggestive of a distinct type of carcinoma or of a distinct type of maturity cells?", in this edition of ACTA CYTOLOGICA).

To Dr. Graham:

In our biopsies we found that the spindle cell surface layer of carcinoma in situ is so vulnerable that previous washing, grasping with instruments or touching during the operation, as well as previous cytological smears, were enough to cause it to exfoliate. The same is true for operation preparations. Therefore, we have used a very careful technique, avoiding all of these possibilities of injury, in order to get untouched material. Sixty-six times in histological sections of 73 cases of carcinoma in situ examined freely, we found a surface layer of spindle-shaped cells with spindle-shaped nuclei (often only in a few from many serial sections). In 87 carcinomas in situ which were further reviewed since this series of investigations, there was again a similar relation. It is remarkable, however, how rarely spindle cells, in comparison, are present in a cytological smear. They are found only in approximately a third of our cases, although according to the histological sections, four-fifths would be expected to have them.

Cell type	Occurrence in the 73 cases each of Ca in situ	of Invasive Ca
Undifferentiated atypical cells	73 X	73 X
Atypical cells resembling basal cells	44	41
Polymorph, immature, atypical cells	55	52
Spindle cells without prekeratin formation	25	20
Tadpole cells without prekeratin formation	22	18
Third type differentiated cells without prekeratin formation	5	1
Spindle cells with prekeratin formation	23	31
Tadpole cells with prekeratin formation	12	19
Third type differentiated cells with prekeratin formation	21	24
Polymorph cells with prekeratin formation	48	49
Parabasal, dyskaryotic cells	49	22
Intermediate " "	53	47
Superficial " " without prekeratin formation	46	24
Superficial " " with prekeratin formation	36	11

The histology of the same cases on the same days showed the following:

Epithelial Layers	with Ca in Situ	Invasive Ca
Basal cell layers	31 X	8 X
Polymorph, plasma-poor (PAS-negative) cell layer	73	73
" " -rich (PAS-positive) " "	69	63
Spindle-shaped surface layer	61	28
Parakertotic layer	2	1

The frequency following various types of tissue proliferation:

Epithelial Layers	with Ca in Situ	Invasive Ca
Polymorph, plasma-poor and plasma-rich cells	7 X	27 X
As above, but with spindle cell covering	35	24
Only polymorph, plasma-poor cells	3	14
All layers: basal, polymorph, plasma-poor and plasma-rich spindle cell covering	21	3
Basal and polymorph, plasma-poor layer	1	2
besides polymorph plasma-rich layer	1	1
besides spindle cell layer and parakeratosis	2	1
Almost exclusively polymorph plasma-rich layer	0	1

Perhaps we cannot distinguish in a histological section whether we are dealing with a real spindle cell or whether the spindle form was only simulated by a cross section through an oval cell which is present in the smear as a dyskaryotic cell, as a tadpole cell or as a third type differentiated cell. I would be glad to hear Dr. Graham's opinion concerning this.

To Dr. de Brux:

I agree with the suggestion of consulting the smear picture for help if an evaluation is not possible from the histological slide alone. I am certain of the agreement of the authors when I maintain that there are spindle cell types which are present only in carcinoma. Others, however, can easily be confused with pseudo-spindle cells of reversible changes if not all cells present in the smear were considered for the final diagnosis.

JORGE CAMPOS R. de C., Lima, Peru:

In cases of carcinoma in situ of the cervix, spindle cells are rarely seen in the cervical or vaginal smears. This type of cancer gives a cytological picture which, although not pathognomonic, in many cases permits the cytologist to state whether or not the cancer is non-invasive.

Exfoliated cells from carcinoma in situ are generally round or oval, medium sized, having clear-cut borders, with scant, cyanophilic cytoplasm and without leukocytic infiltration; the smears have a tendency to be isomorphous, and one does not observe cellular debris.

These cytological characteristics are in accordance with the histological characteristics of the non-invasive cancers. Carcinoma in situ of the cervix is not the same as invasive cancer minus invasion. In carcinoma in situ one does not find pearls, squamous differentiation, cornification or other signs of cellular maturation so common in the invasive cancer. In this respect I disagree with the opinion of Dr. Galbis.

In a series of 50 in situ carcinomas and 12 invasive cancers of the cervix in which the morphological characteristics of 2420 neoplastic cells were studied, neoplastic spindle cells comprised only 3% of the cells in carcinoma in situ cases in contrast to the 19% of neoplastic cells in the invasive cancers (1).

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EMMERICH VON HAAM, Columbus, Ohio, U.S.A.:

I fully agree with Galbis that the presence or absence of spindle cells does not give any information as to the invasiveness of the tumor; but in our experimental production of carcinoma of the cervix in mice, they were found much less frequently as long as the tumor was in the in situ stage, as stressed by Graham. The difference between benign and malignant spindle cells is sometimes quite striking and the most important criterion for this difference seems to be the appearance of the nucleus. In our experiments the observation of "malignant spindle cells" was most commonly associated with established cancer which proved an irreversible lesion even after the carcinogen was withdrawn. I do agree with de Brux that the spindle-shaped squamoid cell represents an abnormal cell and probably assumes its peculiar shape quite early in the process of maturation, but I don't agree with his statement that "the more fiber cells which are present the greater chance there is of the lesion being benign."

JACQUES JENNY and ALFRED WACEK, Zürich, Switzerland:

The vaginal and cervical smears of 85 women with carcinoma in situ, who were admitted to the University Women's hospital, Zürich, were reviewed. Spindle-shaped squamoid cells were found in only 27 cases (32%). They occurred only in the vaginal smear in one patient, only in the cervical smear in 23 patients and in both vaginal and cervical smears in three patients.

When compared with the histological picture of the carcinomatous epithelium, only two of the 27 cases showed an epidermoid structure, whereas of the 58 cases without spindle-shaped cells, 23 showed an undifferentiated type of epithelium.

We would conclude from this that the presence of spindle-shaped squamoid cells suggests in most cases a rather differentiated type of epithelium. There are, however, a number of cases where spindle-shaped cells in the smears are connected with an undifferentiated type of carcinoma

in situ. The absence of spindle-shaped squamoid cell does not allow one to draw any conclusion as to the degree of histological differentiation, since there were a considerable number of cases without spindle-shaped cells, which histologically showed a high to moderately high differentiation.

JULIETA C. de LAGUNA, Mexico, D.F., Mexico:

According to the definition of the Editorial Board we should accept that, although infrequently, it is possible to observe cells with "fibroid" morphology with no malignant characteristics and thus, that they may be present in a benign condition. I agree with Graham on the rarity of fiber cells in carcinoma in situ. Accordingly, I wonder what kind of cells de Brux et al. are labeling as fiber cells in "dysplasias" and in carcinomas in situ.

In our laboratory we also establish our diagnosis based on the general picture of the smear and not only on isolated cells, but we concede a little more specificity to the fiber cell as being a malignant cell, typical of squamous carcinoma.

Concerning Galbis' paper, it must be said that we have probably the same criteria about fiber cell. I disagree regarding his point of view in relation to carcinoma in situ: although carcinoma in situ is a histological fact, in our experience it has been possible to correlate cytological findings with histological appearance and thus make cytological diagnoses of carcinoma in situ or invasive carcinoma. (For figures, see discussion on Symposium A, #14, this issue.)

JAMES W. REAGAN, Cleveland, Ohio, U.S.A.:

Analytical studies of cell configuration in 10,000 cells from 100 patients with histopathologically proven carcinoma in situ indicate that 1.7 per cent of the cells fall into the spindle cell category. Similarly, 1.5 per cent of some 5,000 cells from dysplasia were of this type (1). Since a dysplastic change is observed over the portio in 80 per cent of the women with in situ cancer, the presence of spindle cells in patients with in situ cancer does not necessarily imply that they originate in the focus of in situ cancer. I am of the opinion that they originate more often in the dysplastic change and are manifestations of an abnormal keratinization. A linear fibril can be demonstrated in the cytoplasm of some of the spindle cell forms. This is analogous to the tonofibril and not only may explain the configuration of the cell but also implies a relationship to keratinization since the tonofibril is now regarded as the precursor of keratin.

I am in agreement with the statement made by de Brux et al, that "it is by studying the cellular picture of the environment that one can get some idea of the lesion from which fiber cells are exfoliated." Similarly, I agree with Graham that spindle-shaped cells are observed only infrequently in the presence of carcinoma in situ.

Although it is not pertinent to the discussion, as a pathologist, I would disagree with Galbis in regard to his unqualified statement that "carcinoma in situ is not a dysplasia but a true carcinoma." This is a matter of definition and involves a very controversial issue.

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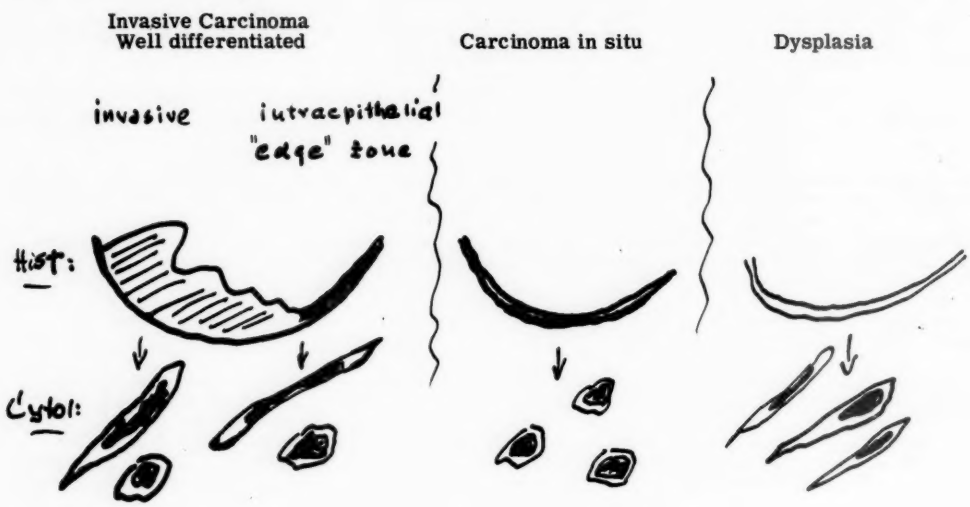
PETER STOLL, Heidelberg, Germany:

This is an extremely important question and I agree entirely with Ruth Graham that spindle cells are very infrequently seen in carcinoma in situ. Our definition of carcinoma in situ is that of a very unripe lesion of the epithelium which should not show cell differentiation in the surface layer. If we see differentiation of the upper cell layer, we would refer to this lesion as dysplasia, as de Brux describes. Dr. Galbis is correct in saying that spindle cells are not seldom in cases of well-differentiated carcinoma, but I would say "invasive or true carcinoma," because for me carcinoma in situ is not a true carcinoma which, if untreated, would progress and definitely kill the patient.

I submit the following thesis: maturation and differentiation are forced upon the squamous epithelium in the invasive lesion as a result of its interrelationship with the connective tissue: stimulants by the mesenchyme (formativer Reiz durch das Bindegewebe). In small carcinomas we see again and again that the carcinoma appears more mature in areas where invasion occurs (pearl formation, monocellular cornification) as compared with its degree of maturation in the surrounding carcinoma in situ. The process of the carcinoma in situ, occurring completely-biologically speaking-in the periphery of the body, is not subject to this stimulus from the connective tissue and, therefore, the carcinoma in situ is always immature.

From this it follows that: if we see a piece of tissue with a carcinoma in situ which seems to be more differentiated, then we always think that we deal here with a marginal area of an invasive carcinoma and try to establish the diagnosis by amputation of the cervix.

This is why we believe that the occurrence of the atypical spindle shaped cells is an indication of a true invasive carcinoma. In these cases we also find other atypical cells in addition to the spindle cells. If other cancer cells are absent, the presence of only spindle cells is evidence of a benign dysplasia.



EDMUND SCHÜLLER Vienna, Austria:

Smears from carcinoma in situ will only very rarely contain spindle-shaped squamoid cells (fiber cells) with all the criteria indicating malignancy, since--according to its histological type--the carcinoma in situ will hardly ever exhibit pearl formation or even attempt pearl formation. My observations are identical with the ones reported by Ruth Graham. I would also like to refer to my own paper in this edition, entitled, "Are Spindle-Shaped Squamoid Cells Derived From the Surface of the Lesion?"

As far as the dignity of the term carcinoma cervicis uteri in situ, a question which has been raised by de Brux and Galbis, is concerned, I would like to say that the terms "carcinoma in situ" and "pre-invasive carcinoma" represent a contractio in adjecto from the standpoint of classical pathology. Whether or not an untreated lesion which is identified as carcinoma in situ will often, usually, or never progress to a true invasive carcinoma no one really knows, despite the rash of publications in which either this or that opinion is fought for with deep emotion.

YOSEUP S. SONG, Providence, Rhode Island, U.S.A.:

It is obvious that the three speakers apparently agree with the fact that the presence or absence of spindle cells in the smears does not indicate any specific type of tumor but only informs us as to the cellular differentiation. As Graham stated, the fiber cells or spindle cells are rarely seen in the cases of carcinoma in situ in our series.

De Brux, Rauzy and Dupré-Froment stated that the fiber cells can be seen in benign lesions as well as in suspected or malignant lesions. I believe these could be interpreted as "benign spindle-shaped cell elements" which can be seen in non-malignant lesions, such as leukoplakia of the cervical epithelium. However, the spindle-shaped cells seen in the benign lesions may not have all the characteristics of malignancy. It would be necessary to clarify the difference between the mature "benign spindle-shaped cell elements" and the true fiber cells.

In reviewing the type of malignant cells seen in 130 cases of histologically proven carcinoma in situ in our series, only one case showed only fiber cells on the vaginal smear (Fig. 1 and 2).

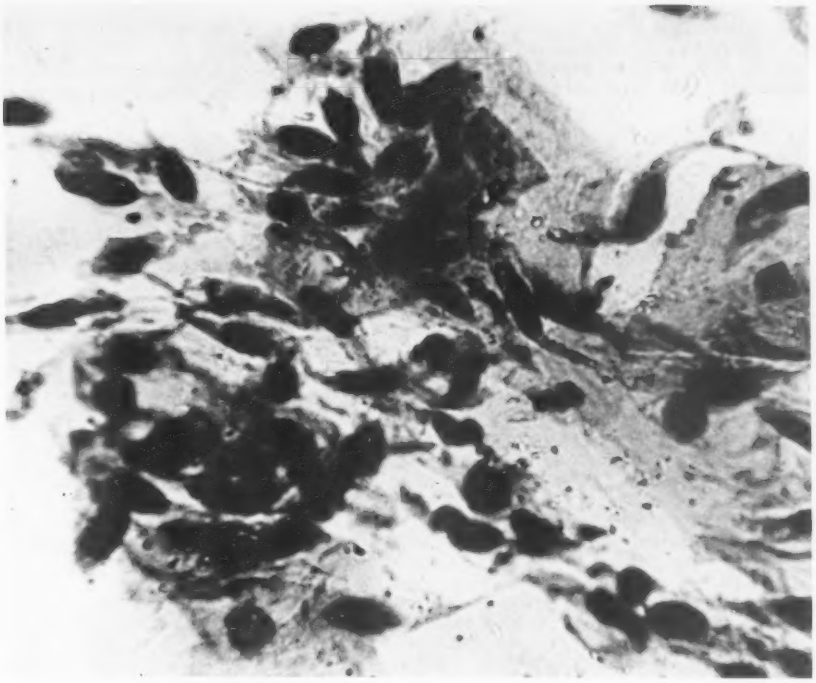


Fig. 1 and 2. Case of a 29-year-old woman. Numerous spindle-shaped cells are seen on the vaginal smears, while the cervical scraping smear was negative. No other type of malignant cells were found on the smears.

This led us to believe that this group of fiber cells apparently originated from invasive carcinom: of the cervix. However, the biopsy revealed an unequivocal picture of carcinoma in situ without stromal invasion. On the very superficial layer of the neoplastic epithelium there are numerous spindle-shaped malignant cells present, some of which are exfoliated (Fig. 3).

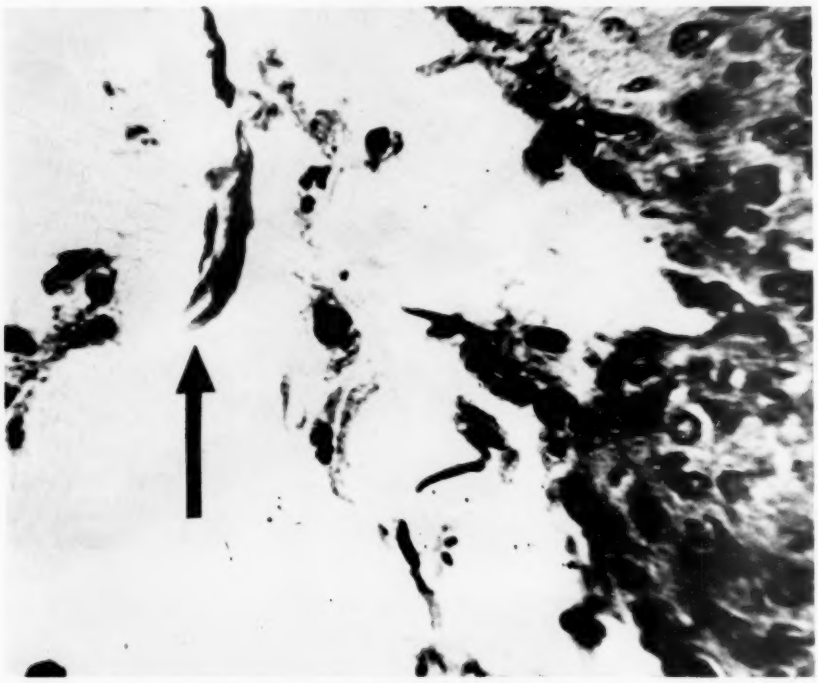


Fig. 3. Cone biopsy section of cervix of the same case. Dx: Ca in situ. Note a few desquamated spindle-shaped squamoid cells.

I agree that these spindle-shaped squamoid cells always arise from an organic epithelial lesion and do not appear in any other circumstances. If the very superficial layer of intraepithelial carcinoma of the cervix is primarily composed of spindle-shaped cells, then the corresponding smear would contain more or only fiber cells, and possibly no other type of tumor cells would be found. On the other hand, in cases of severe leukoplakia of cervical squamous epithelium or active regneration of squamous epithelium in which spindle cells predominate more than in the normal epithelium, the smears would theoretically contain more spindle cells than usual. However, the cells seen in such cases would be morphologically benign, apparently differing from true fiber cells.

CLOSING REMARKS

JEAN de BRUX, Paris, France:

There is great difficulty defining "dysplasia." That is why we attempted to define (a) dysplasia with regular architecture and normal cellular morphology and (b) dysplasia with irregular arrangement and abnormal cellular morphology. Even with this subdivided definition, it is rather difficult to distinguish a "true" carcinoma in situ in the latter group. The criterion to be used is the lack of differentiation or very poor maturation of the cells at the upper level of the epithelium. But, in some cases we found true carcinoma in situ with complete maturity of the cytoplasm, as Drs. Song and Bajardi have reported it.

So, in order to speak about the same cells we must make a clear distinction between the benign and the malignant spindle cells, as Drs. Boschann, von Haam and Song proposed. But in smears of a dysplasia which is suspected to be a carcinoma in situ, one often finds spindle-shaped cells of benign and malignant types together. From these experiences I can explain to Dr. von Haam

my statement, "The more fiber cells that are present, the greater the chance of the lesion being benign." I meant fiber cells of the benign type. As Dr. Reagan said, these fiber cells originate more often in the dysplastic change and are manifestations of an abnormal keratinization " and not necessarily in the focus of in situ cancer."

In answer to the very excellent paper by Horst Smolka, I am astonished that he did not understand my explanations when I said I observed fiber cells in inflammations, because he himself wrote, "I came to the conclusions in these cases that extremely small dysplastic regions (for instance, metaplasias) are likely to be the place of origin." It is just that we mean that the inflammatory reactions of the cervix are always in connection with little ectropions, ectopies and ulcerative areas.

MANUEL GALBIS, Valencia, Spain:

In every discussion there is a certain general agreement about the rarity of the presence of spindle cells in carcinoma in situ. We are completely in agreement with this. Our figure (2.5%) is more or less in agreement with those figures of Campos and Reagan.

Actually there are both malignant spindle cells and cells of "fibrinoid" morphology, which, as Dr. Laguna says, are without definite characteristics of malignancy. These two types are not always easily differentiated. Dr. Song remarks the necessity of distinguishing between the "benign spindle-shaped cells and the true fiber cells." The true fiber cells with the characteristics of malignancy are very rarely seen in carcinoma in situ; on the other hand, Reagan correctly states that in a carcinoma in situ, spindle cells can be found, but without necessarily having their origin in the neoplastic area.

Reagan has reason for maintaining that it is not pertinent to discuss the concept of carcinoma in situ here, but perhaps the translation has not expressed my meaning. When I say that the carcinoma in situ is not a true concept, I mean that these histological diagnoses are only made when the cellular atypia are morphologically similar to that of carcinoma, but without saying anything additional.

The true malignant spindle cells are probably a sign of cellular differentiation. It is true that such differentiation is not usually observed; however, it is illustrated below in the microphotograph (Fig. 1) in the superficial layer of flat fibroid cells. The smears from this patient showed abundant spindle cells. In this respect, the opinions of Stoll are interesting, but I do not think that we must rule out the possibility of true spindle cells in carcinoma in situ.

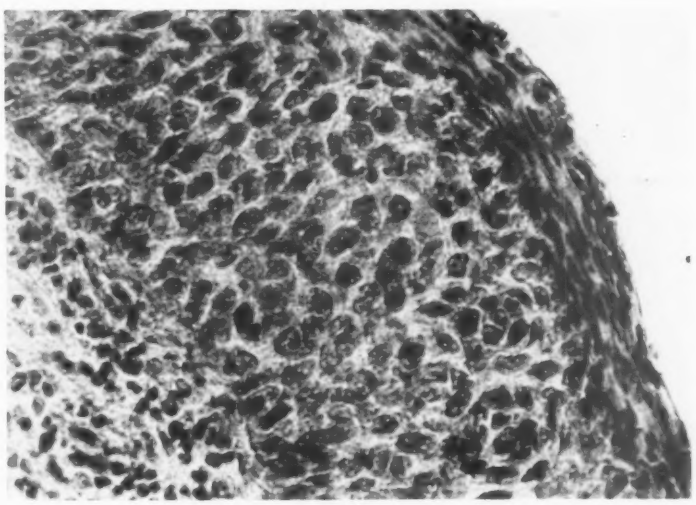


Fig. 1

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

There seems to be rather good agreement that fiber cells occur infrequently in cases of carcinoma in situ of the uterine cervix. The major point of disagreement seems to be whether the

absence of fiber cells indicates a carcinoma in situ or the presence of fiber cells an invasive lesion. It is my impression that such a definitive diagnosis of the extent of the lesion by the presence or absence of fiber cells is demanding that the cytologic method do more than is actually required of it. The case cited by Song is a good illustration of the difficulties inherent in trying to predict whether or not the basement membrane has been invaded by looking at surface cells.

Boschann's observation that if great care is taken in securing the histologic specimen, fiber cells will occur on the surface in four-fifths of the cases is of great interest. I, too, have been impressed with how often one sees a surface layer of cells which resemble fiber cells in the histologic section of a carcinoma in situ. Yet they are found so infrequently in the cytologic preparation. Such a discrepancy between the histologic and cytologic might be accounted for by a difference in the rate of desquamation of these elongated malignant cells. This is suggested by the figures of Jenny and Wacek, in which they state that in only four out of eighty-five cases were fiber cells found in the vaginal smear while 32% of the cases had fiber cells on the direct cervical smear. Since Boschann reports fiber cells in 33% of the cases of carcinoma in situ, it would seem to indicate that they are not seen as frequently when only naturally exfoliated cells are examined. Unfortunately the problem is not as simple as this since Reagan uses cervical scrapings and has found only 1.7% of the malignant cells to be of the fiber cell type.

Probably a more important factor than the rate of desquamation is that we are trying to correlate here the cytologic picture with a histologic picture which is not interpreted the same by different observers. When I said above that I have many times seen cells, which resemble fiber cells, on the surface in the histologic section, I did not mean that if this picture alone were present the diagnosis would have been carcinoma in situ. It has been my experience that most pathologists will not consider a lesion to be a carcinoma in situ unless the entire layer is composed of immature cells, and if the surface is extremely well differentiated it is apt to be placed in a different category. This brings up an interesting point since Boschann suggests that these surface cells are lost through trauma to the specimen.

This discussion shows again how difficult it is to correlate cytologic material with a lesion such as carcinoma in situ where there is so much disagreement from the histological viewpoint. It is encouraging that we all agree so definitely that these fiber cells rarely occur in this lesion, even though there may be some disagreement as to the significance of this finding.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

OCCURRENCE OF SPINDLE-SHAPED SQUAMOID CELLS IN INVASIVE CARCINOMA

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

In contrast to the cytologic picture in atypical epithelium and in carcinoma in situ, fiber or spindle cells are a common component of invasive squamous carcinoma. In sixty cases of Stage I squamous carcinoma of the cervix, forty-two or seventy per cent had fiber or spindle cells in the vaginal smear. If one considers cases of invasive carcinoma, all stages of disease, seventy-two per cent of an additional hundred cases had fiber cells. It would appear that when the cancer is invasive, over two-thirds of the cases will have fiber or spindle cells.

Though fiber cells occur in invasive squamous carcinoma, they are by no means very common. In only four cases were spindle or fiber cells more than 50% of the total malignant cell population, and in a little over half the cases they were less than ten per cent. These figures are based on surface exfoliation, since the cytologic material consisted of vaginal smears. It is possible that if scrapings were done fiber cells might be present in larger numbers.

JULIETA C. de LAGUNA
Mexico, D.F., Mexico

In our experience, "fiber cells" are pathognomonic of squamous carcinoma either of the cervix or of some other location. They permit the identification of a definite type of tumor as well as the identification of the degree of differentiation. Such a "fiber cell" morphology is evidence of a particular stage of the process, dependent on a given metabolic situation. In other words, we do not think that its morphology has any relation to its location in depth in the tumor.

Although, as is common knowledge, some squamous carcinomas show no "fiber cells," in a survey of more then 2000 carcinomas of the cervix, we have been able to confirm a cytological finding that correlates regularly with the pathological diagnosis of squamous carcinoma, Broders Grade II: In the absence of cellular monstrosities, the criterion is the presence of "fiber cells," regardless of their number.

Another fact is that the presence of a maximal degree of atypia corresponds to a higher degree of histological undifferentiation (Broders Grade III or IV) and this we have confirmed in our routine practice of grading squamous carcinomas of the cervix, which we have carried out since 1951. So far, an 80% correlation between our degrees and those of pathologists have been obtained.

In carcinomas with area of higher undifferentiation there are exfoliated cells showing more marked nuclear abnormalities.

We have never been able to observe "fiber cells" in carcinoma in situ and, accordingly, their presence, like the presence of some other types of cancerous cells (spindle cells, tadpole cells, etc.), allow us to make a diagnosis of invasive carcinoma.

HORST SMOLKA
Kiel, Germany

Typical spindle cells are comparatively rare in the cytologic smear in invasive squamous cell carcinomas of the cervix. They may occur in cancer tissues of different grades of ripeness and are not always the expression of highly differentiated or keratinized carcinomas. They do not prove the predominant histologic pattern of ripeness of the tumor, for parts of tissue consisting of spindle-shaped cells can be observed side by side with parts composed of cells of large irregular forms or of smaller, round, more embryonic types. It is not always easy to recognize spindle-shaped cells in the tissue. Often they are indicated only by regularly elongated shapes of the nuclei and the densely stratified growth pattern of the tissue.

Orange or red staining is more a sign of a higher differentiation than blue or greenish (Papanicolaou or Shorr stain). However, the orange color can also be due to hemolized blood impregnating the cells.

The shape of the cell depends on the state of the cytoplasm. Differentiated cells generally show distinct outlines, while in undifferentiated cells the cytoplasm is hazy and the cell outlines are indistinct; the cell looks like a fine brush stroke.

There are often transitional forms between spindle cells and fiber or snake cells. Therefore, a clear definition of the cells as one of these forms is not always possible. The cell can be extremely long so that occasionally its length may be 30-40 times its average width. The length may be 150μ or more. The long body of the blunt-ended snake cell may be wound like a narrow ribbon. Sometimes small vacuoles are observed in the cytoplasm of the spindle and snake-shaped cells.

The nuclei may have different shapes; very frequently they are oval (or elongated) and hyperchromatic, sometimes to such a degree that no structures in the nucleus can be recognized. However, the appearance of the nuclei may also occasionally be lighter, but in these instances they show irregularly granulated structures. In most of the spindle cells originating from invasive squamous cell carcinomas, the nucleus occupies the whole width of the cell. Frequently the nucleus even exceeds the cell width, the cell border thus forming an arch. The width of the nucleus is limited by that of the cell, while its length is occasionally considerable; the nucleus itself may look like a long rod or a spindle. Often it is not possible to recognize nucleoli due to strong hyperchromasia or irregular structures. Multinucleation is observed rather frequently.

The number of spindle cells found in smears differs considerably and depends entirely on the tissue structures of the carcinomas. In many cases only one, or very few, cells will be found. Only occasionally can loose groups of spindle cells be observed, and it is only in rare cases that they predominate throughout the smear.

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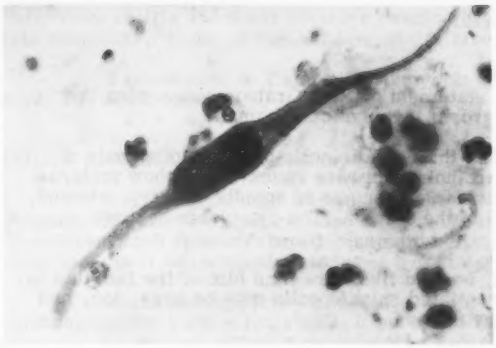

Fig. 1. Spindle-shaped cell with two hyperchromatic nuclei. (Horst Smolka)

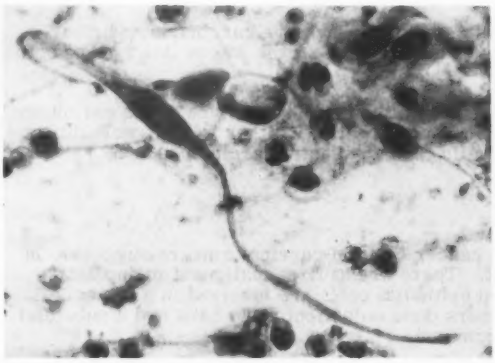

Fig. 2. Snake cell. (Horst Smolka)

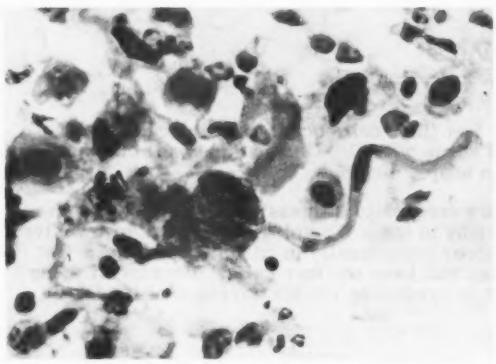

Fig. 3. Snake cell among numerous abnormal cells of different forms and structures. (Horst Smolka)

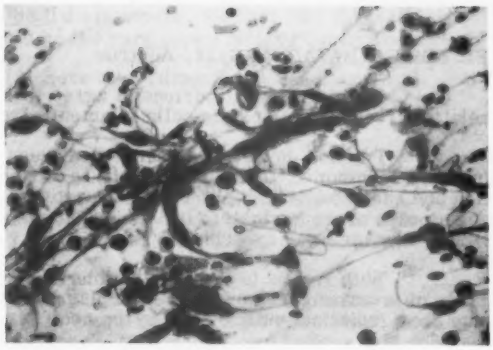

Fig. 4. Numerous filiform cells in a predominantly unripe carcinoma. (Horst Smolka)

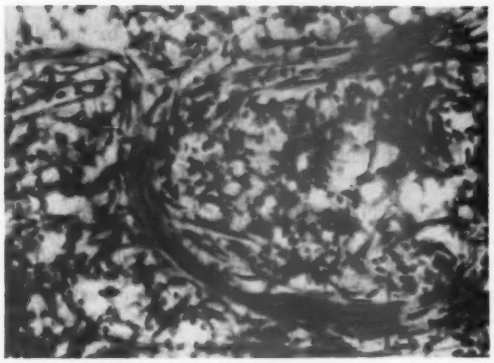

Fig. 5. Dense groups of spindle cells surrounded by blood. (Horst Smolka)

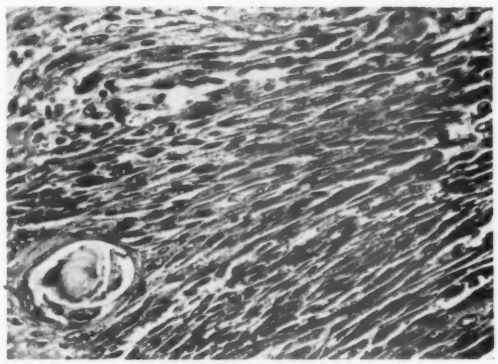

Fig. 6. Same case as in Fig. 5. Histologic section of a differentiated carcinoma with partial tendency to keratinization. The spindle-shaped cells can also be recognized distinctly in the tissue (alcohol fixation, Hematoxylin-Eosin stain). (Horst Smolka)

DISCUSSION

FRIEDRICH BAJARDI, Graz, Austria:

From my own experience I agree with the statement of Ruth Graham concerning the relative frequency of spindle cells in smears from (gross) invasive carcinomas.

I do not, however, agree with Laguna's view that spindle cells permit a diagnosis of invasive carcinoma. I could demonstrate surface carcinomas in rare cases which show maturation and can exfoliate spindle cells. Moreover, a surface carcinoma of spindle-cell type showed, as one case proved, clusters of spindle-shaped cells in the smear. The cytoplasm of these cells, however, was cyanophilic.

With regard to the explanations of Smolka, I would like to remind him of the fact that in cases of invasive carcinomas the cytoplasm of differentiated spindle cells may be hazy, too, and may show indistinct outlines due to the necrosis of the tumor.

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JORGE CAMPOS R. de C., Lima, Peru:

It is common to find fiber or spindle cells in smears of invasive carcinoma, whether from the vagina or from other sources; but it is very unusual to find a case where this type of cell predominates in the smear.

The presence of these cells depends much more on the histological type of the carcinoma than on the location within the epithelium layer of the exfoliated cell. In other words, well-differentiated carcinomas are more likely to exfoliate fiber cells than anaplastic carcinomas.

LUBA A. TURNBULL, Montreal, Canada:

The presence of spindle-shaped squamous cancer cells in cervical smears suggests, in my opinion, invasive or clinical cancer of the cervix. There are at first malignant metaplastic cell changes that occur in some cases where the deep epithelial cells are involved in a tumor. It is not rare that these can be detected in cervical smears done on patients who have had a sub-total hysterectomy; in other words, in cervical stump carcinoma.

In the 21 cases of squamous carcinoma of the cervical stump that I have had the opportunity to observe in our laboratory and that had been confirmed pathologically as invasive carcinoma of the cervix, 9 cases showed spindle-type cancer cells in the smear.

One of the 9 cases showed spindle-shaped cancer cells one year after sub-total hysterectomy had been performed.

CLOSING REMARKS

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

There seems to be rather good agreement that fiber cells occur rather frequently in invasive squamous carcinoma of the cervix. I would agree with Dr. Campos that well differentiated tumors are more likely to shed fiber cells than anaplastic ones.

Though it is true that fiber cells occur more frequently in invasive carcinoma than in carcinoma in situ, I feel that it would be somewhat risky to make a cytologic diagnosis of invasive carcinoma on the presence of fiber cells. They do occur occasionally in carcinoma in situ. On the other hand, about one third of invasive carcinomas will have no fiber cells. Because of these two facts there will be rather a wide margin of error in predicting the invasiveness of the lesion.

JULIETA C. de LAGUNA, Mexico, D.F., Mexico:

I agree that spindle cells are not the most common morphological type in squamous carcinoma. Nevertheless, in our experience they are the most characteristic cell type, and when they occur in the smear in large numbers (not associated with other cells with marked nuclear monstrosities), histological differentiation in such cases corresponds to Broders II; in this condition we feel that we can correlate the presence of spindle cells with the degree of differentiation of the tumor. They have been, in our experience, the best sign for the differential diagnosis between

carcinoma in situ and early invasive carcinoma. As stated elsewhere, we have had 15% error in this diagnosis. Some of these diagnostic failures could be the rare cases pointed out by Bajardi.

I agree with de Campos's comments, and I believe that Turnbull, in those aspects, holds the same opinion.

HORST SMOLKA, Kiel, Germany:

I fully agree with Bajardi that the occurrence of spindle-shaped squamoid cells is not a reliable indicator for the presence of invasive carcinoma. Regarding the borders of the cytoplasm of differentiated spindle-shaped squamoid cells, I did not deem it necessary to mention that necrotic cells lose their shapes and structures by autolysis.

As to the frequency of spindle-shaped squamoid cells in invasive carcinoma as mentioned by discussants, I am astonished. It seems to me that this frequency has to be considered as relative when compared with non-malignant cellular alterations. Apart from this I believe that conclusions regarding the presence of spindle-shaped squamoid cells are often based on histological pictures. One finds, however, that one cannot always distinctly discern the real shape of the single cell in histological sections.

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ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

OCCURRENCE OF SPINDLE-SHAPED SQUAMOID CELLS IN EXTRA-GENITAL CARCINOMA

RUTH M. GRAHAM
Buffalo, New York, U.S.A.

"Spindle cell" has been used in this discussion to indicate a malignant cell with an elongated configuration but a shorter and broader form than the "fiber cell." This distinction is a helpful one, particularly in non-genital carcinoma. In squamous carcinoma of the lung, spindle cells are fairly common, but true fiber cells are rare. It has been my experience that the fiber cell occurs in squamous carcinoma of the cervix much more frequently than in squamous carcinoma of other sites. I once saw a positive sputum which had great numbers of fiber cells, but the lesion in the lung was metastatic from the cervix. Spindle cells may be found in carcinoma of the esophagus frequently but again true fiber cells are rare. I have not had enough experience with cancer of the oral cavity to know whether fiber and spindle cells occur. Perhaps some of the discussants have had experience in this field.

HEINZ GRUNZE
West-Berlin, Germany

I have been asked to tackle the question of the specificity of spindle-shaped squamoid cells for the diagnosis of carcinoma. From some discussions I had with gynecological cytologists it appears that considerations of this kind have been made by gynecologists. As far as pathological alterations outside the genital tract are concerned, the question has to be answered negatively, even if we distinguish sharply between "spindle cells" and "pseudo-spindle cells" as the gynecologists do. Any other idea would be liable to cause astonishment. For this would mean that the "spindle cells of squamoid type" would provide the first absolute qualitative cytological criterion of malignancy. So far, however, it appears—theoretically as well as practically—that the morphological qualitative criteria for benign and malignant growths are the same. They can only be distinguished by the degree of alteration, which is based on observation of large numbers of cells using statistical evaluation. The individual cell thus may always belong to a range of alteration indifferent for diagnostic purposes.

In order to illustrate this basic rule of interpretation, not only spindle-shaped cells but also other atypical squamoid cells which are also usually regarded as suspicious for cancer will be used. In a cytological sample, for instance, the "spindle cell" shown in Fig. 2a was found. We believe that the other cells, Figs. 2b-2e, found in the same sample would have been as suspicious as 2a.

Spindle-shaped cell elements can be seen in non-carcinomatous alterations, for example in epidermoid metaplasia arising from chronic inflammation in the bronchial tree or in chronically altered epithelia of the oral cavity. My own experience so far has convinced me that in the case of benign spindle-shaped elements, all processes of hyperkeratinization of the cytoplasm can be present, as well as nuclear pyknosis and marked enlargement of the nucleolus. Only the frequent rather impressive nuclear hyperchromasia of tumor cells was missing almost regularly in these benign cases. Unfortunately, however, this criterion of differentiation cannot be trusted, as even in carcinoma the degree of hyperchromasia shows marked variation and can be of very little intensity in single cells.

The occurrence of spindle-shaped squamoid epithelial cells seems to depend on the existence of a free surface of the tumor. For, only under these circumstances did I find sharply outlined "spindle cells." In samples from lymph nodes or organ punctures (Fig. 3a, b) "spindle cells" are much less frequent than in samples from surface carcinomas. Furthermore, the "spindle cells" in these punctures hardly ever show any sharp borders. Some of the spindle-shaped squamoid cell elements found in solid tumors may have been caused only artificially by distensions and disconfigurations due to the smearing technique.

It seems justified to state that all surface alterations exhibit a much stronger tendency to form spindle-shaped cell elements than organ tumors do. It is my experience that spindle-shaped cell elements inside an epidermoid carcinoma only originate under conditions of central degeneration or even dissolution.

In my opinion, some substantial doubts have to be directed against the term "spindle cell," since it may be very misleading to use the term "spindle cells" in reference to squamoid carcinomas, as this term has long been applied by pathologists to morphological elements characteristic for fibroblastic sarcomas. Under these circumstances I should prefer to say "spindle cell-like." The correspondence preceding this symposium showed some attempts to obtain a clear and uniform terminology, although it is lacking so far.

Indeed, in smears of sarcomas "spindle cells" occasionally are found, as is demonstrated in Fig. 4b for differential diagnostic purposes. They occur, however, much less frequently in the smear than in sections of the same tumor (Fig. 4a). This difference in the number of "spindle cells" between the smear and the section demonstrates how much was due to the technique. These cells as well as their benign variants in the connective tissue show a rather torn-up cytoplasm with indistinct borders. Different from epidermoid carcinomas, sarcomas thus demonstrate the coherency of the individual cell to the total tissue. In Pappenheim staining the cytoplasm shows a gray color with, eventually, a weak green component, while in Papanicolaou staining the gray color eventually tends to develop a weak pink shade.

This we consider a useful criterion for the differentiation of sarcoma cells from sharply bordered "genuine spindle cells of squamoid epithelial origin" (according to the terminology of gynecological cytology). In our experience "spindle cells of squamoid epithelial origin" always showed a non-transparent red or orange cytoplasm with Papanicolaou staining and a shining blue with Pappenheim staining.

In summary, spindle cell-like elements are found also in extragenital localizations of squamoid epithelial alterations, often in carcinomas, seldom in benign changes. The latter must derive from chronic inflammations of severe degree if their morphology is so intensively altered that they may be confused with similar cells originating from carcinomas. This seldom occurs. The individual spindle cell as a singular finding, therefore, cannot be regarded as an absolute criterion of differentiation between benign and malignant alterations.

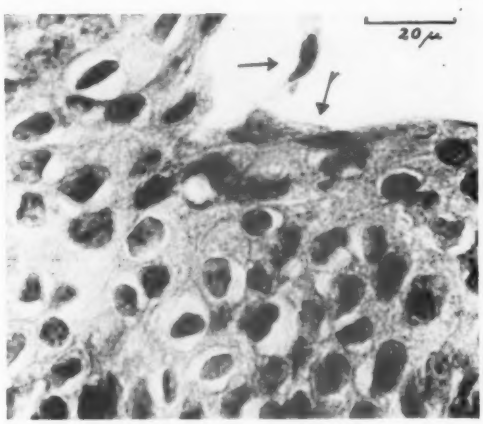


Fig. 1: H.E. stain. Section. Epidermoid metaplasia in draining bronchus of a chronic pulmonary abcess. In spite of the markedly enlarged germinal zone at the surface, a small area with a spindle cell-like pattern can be seen (↙). Exfoliated spindle cell-like element (↙).

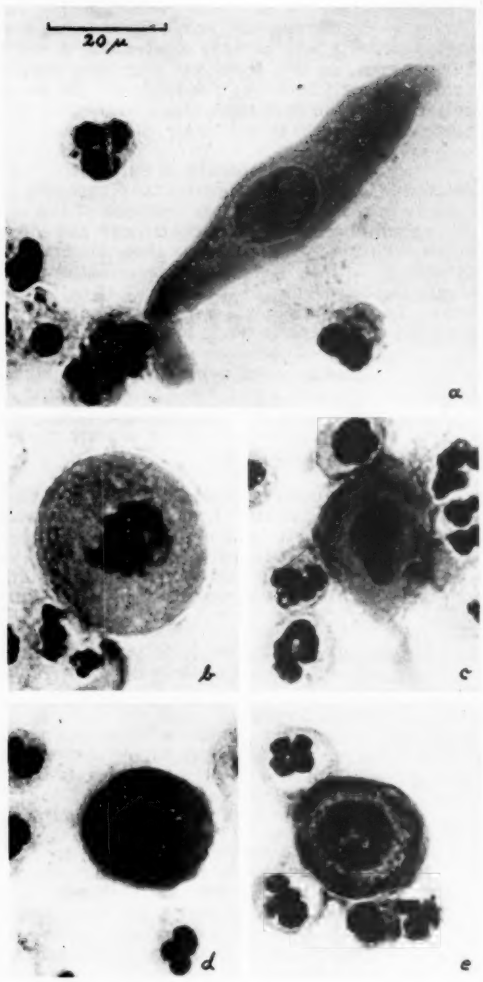
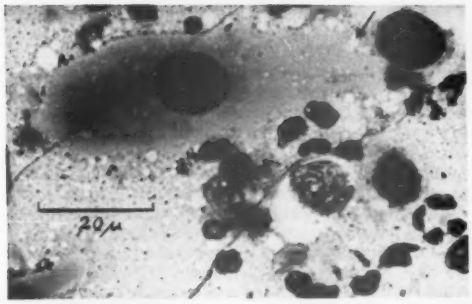


Fig. 2 a-e: Papanicolaou stain.
Cytological sample of the tumor sectioned in Fig. 1
All cells can be seen to exhibit marked keratinization, partly of a diffuse (a,b,c,d) and partly of a granular (e) type. The cytoplasm with Papanicolaou stains orange. While b, c, d and e show marked nuclear hyperchromasia and a more or less severe pyknosis, in b even karyolysis, the nucleus of the spindle cell-like element still appears loosely reticular and shows a prominent nucleolus. According to the very mature cytoplasm of this cell, there is a clearly recognizable dissociation of maturation, a finding more frequent in carcinomas.



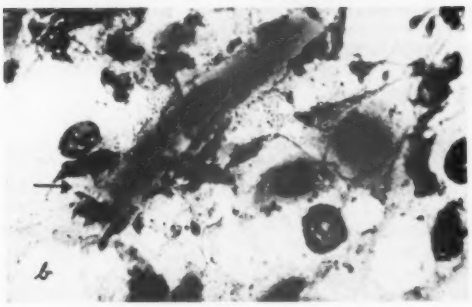
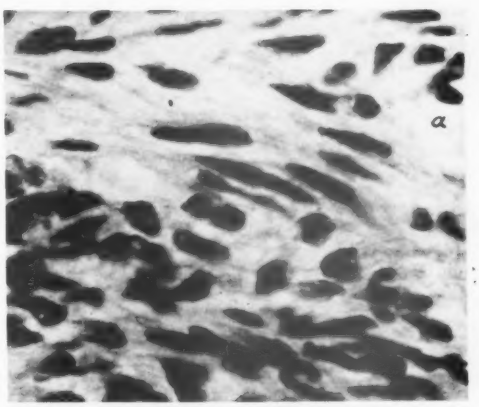


Fig. 3 a,b: Pappenheim stain.
Lymph node puncture.
Cornifying epidermoid carcinoma with spindle cell-like elements.
The cell borders are not as sharply outlined (↙) as in cells of exfoliative samples. Distinct nuclear hyperchromasia and beginning pyknosis in b. The cytoplasm in the Pappenheim stain appears shining blue; in the Papanicolaou stain, orange or red.



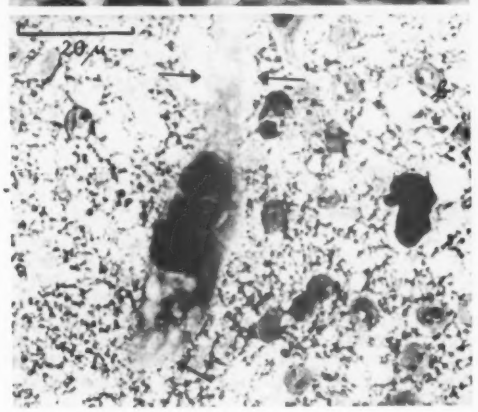


Fig. 4 a,b: Pappenheim stain.
Spindle cells of a fibroblastic bone sarcoma.
H.E. Control section: Dr. Günter Pahlke.
Identical degree of magnification in both figures. Apparent shrinking in a. The torn-up cytoplasm with ill-defined cell borders (↙) occurs in sarcomas frequently. The same, however, can also be found in punctures of benign alterations of connective tissue. The nuclear hyperchromasia in b can be recognized easily.

DISCUSSION

EMMERICH VON HAAM, Columbus, Ohio, U.S.A.:

Grunze emphasizes the fact that there still does not exist an absolute qualitative cytological criterion of malignancy and that this diagnosis still must be made upon the observation of a large number of cells with statistical evaluation of the various degrees of alterations. Later he states that the nuclear hyperchromasia of tumor cells was missing "almost regularly" in the benign atypical spindle-shaped cell elements. In my cytological interpretation of needle biopsies, I have found many spindle-shaped cells in lymph nodes of metastatic transitional cell carcinoma of the neck and spindle-cell carcinoma of the thyroid which did show sharp borders and did not represent artifacts due to the smearing technique since only imprints were used for the study. For this reason, I cannot agree that the occurrence of spindle-shaped squamoid cells depends only upon the existence of a free tumor surface. Typical spindle cells are also the so-called polyp cells of the papillary polyp of the colon. And, of course, there are the spindle-cell sarcomas to which the author refers in his essay. I do agree with him that the singular finding of individual spindle cells cannot be regarded as an absolute criterion of differentiation in benign and malignant alterations. With reference to Graham's remarks, I would like to state that there are indeed benign and malignant spindle cells or even fiber cells which can be differentiated from each other principally by nuclear changes present.

LEOPOLD G. KOSS, New York, New York, U.S.A.:

This discussor is somewhat handicapped by the unavailability of illustrations of Grunze's paper and by the poorly defined topic. Obviously, as Grunze pointed out, elongated, "spindle" cells are not the exclusive feature of squamous cancer, but may also occur in a variety of benign and malignant tissues. Sarcomas, incidentally are not the only tumors that might be composed of spindle cells, but a variety of epithelial cancers of various origins may produce this cell form, just to mention the giant-and-spindle cell variety of thyroid carcinomas. Another point aptly brought up by Grunze, is the difference in the appearance of the tumor in tissue sections and in cytological material. It is quite true that tumors appearing "spindly" in sections may shed cells of all kinds of shapes, but the reverse may also be true, namely, that tumors appearing to be composed of round cells in sections, may appear "spindly" in cytologic material, especially when given the opportunity to grow freely as in pleural or ascitic fluid. An example of the latter transformation is the neuroblastoma which in body fluids nearly always gives elongated, spindle cells.

It is not always possible to recognize spindle cells of squamous origin from those of other origins. The staining qualities may fail completely and the eosinophilic quality of the cytoplasm may be misleading. As a rule of thumb, I would say that squamous cells of all shapes aspirated from sites where no squamous epithelium is normally expected, nearly always signify carcinoma. As far as squamous cancer in loco nascendi is concerned, the same rules of cytologic differential diagnosis apply as to the cervix. Occasionally, the mucosa of the buccal cavity and the larynx will shed benign spindle-shaped squamous cells, but they are extraordinarily rare. This discussor has never seen any convincing evidence that benign spindle, squamous cells ever originate in the lower respiratory tract and would a priori regard with suspicion any such cell labelled as "metaplasia."

The point of differentiation between spindle cells and fiber cells is of great interest, but I cannot contribute in this direction.

In summary, if a spindle cell can be identified as being of squamous origin outside the female genital tract, such a cell should be regarded with suspicion and evaluated according to the cytologic context. One does not make the diagnosis of cancer on one cell, but one cell may be helpful in finding other cells.

YOSEUP S. SONG, Providence, Rhode Island, U.S.A.:

Those spindle-shaped cells derived from non-carcinomatous epithelial alterations can be designated as "benign spindle-shaped cell elements" in order to clarify the definition of spindle-cells as indicated by Graham, who stated that the "spindle-cells" are malignant squamous cells characterized by elongated configuration but a shorter and broader form than the true fiber cells. These "benign spindle-shaped elements" are quite frequently observed on the smear taken from the oral cavity of patients seen at the tumor institute as part of routine physical check-ups. The cells are elongated with broadened cytoplasm, but the nuclei appear to be benign. These cells seem to be derived from the benign lesions of the oral cavity such as leukoplakia of the oral mucosa and tongue and certain cases of hairy tongues. On the other hand, the true fiber cells derived from squamous cell carcinoma of the tongue and oral mucosa are infrequently observed on the smears. On this point I agree with Graham that true fiber cells occur much more frequently in carcinoma of the cervix than in squamous cell carcinoma of the tongue and oral mucosa.

Malignant spindle-cells were rarely seen in vaginal smears taken from patients who had extra-genital carcinoma which had metastasized into the uterine tubes or ovaries without involving the uterine cavity itself.

Figure 1 represents spindle cells seen on the vaginal smear taken from a middle-aged woman who had a primary transitional cell carcinoma of the renal pelvis with an extensive abdominal carcinomatosis. Both of her adnexae were involved by tumor, but the uterus was free from invasion. Vaginal smears showed a great many spindle-cells, but no other type of malignant cells were seen.

The true fiber cells were also rarely recognized on vaginal smears taken from patients who had extra-genital squamous cell carcinoma which had metastasized to the adnexae without penetrating the uterine cavity. The vaginal smears taken from a 39-year-old patient who had primary squamous cell carcinoma of the rectum which had extended directly into the left adnexa revealed a great number of fiber cells and many undifferentiated tumor cells. No tumor was found in the uterine cavity, but the section of the left tube revealed massive invasion of the tumor on the tubal mucosa.

I have no comments on the occurrence of spindle-cells in sputum and bronchial washing smears because my experience in this field is very limited.

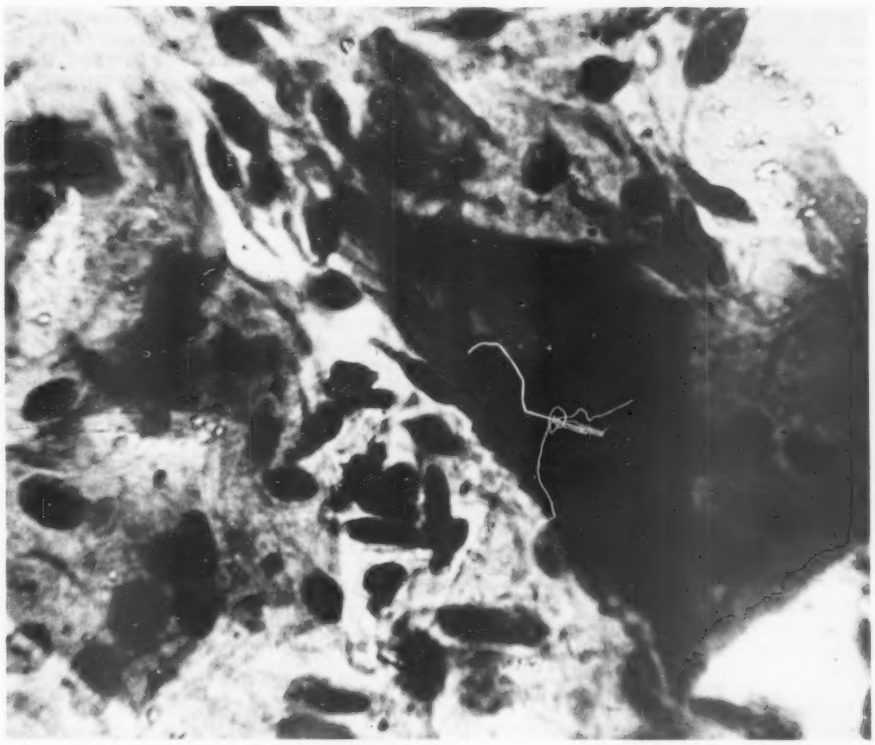


Fig. 1: Papanicolaou staining. Vaginal Smear.
Quite a few spindle-shaped squamous cells are seen in a vaginal smear, having orange colored cytoplasm and spindle-shaped hyperchromatic nuclei. Primary transitional cell carcinoma arising from renal pelvis with an extensive metastasis. Uterus was free of tumor invasion.

CLOSING REMARKS

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

I would agree with Koss that the finding of atypical spindle cells in an area where squamous epithelium does not exist should be looked on with great suspicion and a diligent search should be made for other abnormal cells.

I agree with von Haam that the finding of spindle cells does not depend on an exfoliating surface. A good example of this is in lymph node impressions in carcinoma of the vulva. The positive nodes in well differentiated squamous carcinoma of the vulva may have many typical malignant fiber or spindle cells.

I am pleased to have von Haam emphasize that there are both benign and malignant cells with a spindle or fiber shape, but that they can be differentiated from one another by the nuclear changes present.

HEINZ GRUNZE, West-Berlin, Germany:

The experience of Dr. von Haam of Columbus, Ohio with the examination of metastatic lymph nodes of the neck and spindle cell carcinomas of the thyroid (an expression which is not common with us) is very interesting to me. I would like to see the pictorial documentations his statements are based upon. Also, I would like to see whether the changes he found in these cases bear this spindle cell characteristic or whether we are dealing only with cells in which the ratio of length to width just reaches the ratio which was mentioned in the beginning of the symposium in the definition of spindle cells.

In addition, I would like to agree with Dr. von Haam's closing statement referring to Dr. Graham. It is also my opinion that, as a rule, by means of experience and total observations of the smears, one succeeds in distinguishing malignant from benign changes. It is especially possible to recognize the cells originating in malignant tumors when the nuclear changes are well pronounced. However, there should not be any doubt that there are also carcinoma cells in which these nuclear changes are not present in such a marked way.

My remark concerning the application of statistical methods in the evaluation of measurements or qualitative differences between cells coming from malignant and benign changes perhaps has been misunderstood. It should not have been stated that we consider statistical methods in the diagnosis of a single case necessary. It should merely be brought out that the diagnosis of an individual case is based upon steadily growing personal experience, which places the border line cases in the foreground. In the smears where only a few cells are present to be evaluated, and where these are uncharacteristic in their changes, every cytologist will be confronted with the statistical in-between area. Fortunately, this situation is very rare; however, it appears to me to be of principal theoretical importance.

With regard to the very interesting statements of Dr. Koss, I must, as he, express my regrets that my microphotographs have not been available for the discussion. Everyone of us knows how insignificant the caption of a picture is, compared to the picture itself. I would like to agree with the thesis advanced by Dr. Koss. Also it appears to me highly suspicious when I find spindle-like forms in an area where squamous epithelium does not exist under normal conditions. However, it must be stated that until now I have seen very marked gradual changes 3 times in metaplasia—twice in lung abscesses, once in lung TB (as they are discussed in my paper). In all three cases, the operative specimen was examined in serial histological sections. Two experienced pathologists, independent of each other, formed the opinions that they were dealing merely with a very severe metaplasia.

I demonstrated in one of the previously mentioned cases not only the spindle cells, but also the other qualitative changes. I intentionally did so, since I consider it very questionable and very dangerous when someone selects just a characteristic of a certain type of carcinoma and discusses it. At least in subconsciousness the thought always arises that there might be some qualitative microscopical and morphological characteristics which per se prove malignancy, independent of their quantity. Especially in physicians not educated in cytology, there is still uncertainty with regard to these problems. To prevent mis-understandings in this direction, I did not discuss only the spindle cells.

The previous statements also refer to our principal attitude toward the nomenclature cited by Dr. Song. In addition, we do not like to speak of malignant or benign cells, nor do we like to speak about malignant or benign spindle-like cells, because not every cell originating from a malignant tumor shows the amount of clinical malignancy by its gradual qualitative changes. The conception of malignant is, in the first place, a clinical-functional one for us. Just recall the apparent morphological harmlessness of certain uniform, small, cellular, round cell sarcomas, as it is demonstrated in the cytological smear in comparison with the entirely different severe,

clinical malignancy. For these reasons, we prefer to speak of cells which either come from malignant tumors or from benign changes.

This difference leaves open the possibility that single cells can occur also in malignant changes, which, may be confused with changes of benign origins, perhaps because of their degenerative stage or because of the certain metabolic phase in which they are, at least temporarily, relatively harmless looking. However, this particular cell would still be a "malignant cell," that is, one belonging to a malignant tumor.

The reason that our opinions partially differ may be due to the fact that our methods and the experiences gained therein are different from those in the United States, partly because we lack sufficient funds for a large laboratory and have to work in hospital laboratories used mostly for routine purposes. It is not surprising that our clinical point of view is sometimes over emphasized and that the main significance of a single case is sometimes over estimated.

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ARE SPINDLE-SHAPED SQUAMOID CELLS SUGGESTIVE OF A DISTINCT TYPE OF CARCINOMA OR OF A DISTINCT DEGREE OF CELLULAR MATURITY?

EDWARD E. SIEGLER
Garfield Heights, Ohio, U.S.A.

One must answer the first part of this question from two approaches: First, using the cytological diagnosis as the starting point, we found that in a group of 57 cytologically suspicious or positive for cervical carcinoma in situ smears, in only 3 instances (5%) was invasive carcinoma diagnosed by tissue. When the cytological diagnosis was either suspicious or positive for invasive carcinoma in 42 cases, 13 cases or 31% were only in situ cancers. The total percentage of misleading cytological diagnoses in this tissue proven cancer group of 99 cases was 16%.

Secondly, in a recent group of 126 in situ carcinomas, fiber or spindle cells were described in only 13% of the smears. In an invasive group of 63 cases, 70% of the smears had spindle cells. Therefore, using only the spindle cell as the differentiating point, i.e., their presence implies invasion, their absence in situ cancer, we would fare almost as well as interpreting the total cytologic picture.

Our most helpful criteria in the total cytological picture interpretation of in situ carcinoma are these: the absence of fibers on a positive slide which has a clean background and whose predominating positive cells occur as single cells of the uniform malignant basal cell type. On the other hand, when we see a positive smear with or without fibers, but which also has large clusters of poorly differentiated tumor cells whose nuclei vary greatly in size and shape and whose background shows evidence of necrosis, we suggest the cytological impression of invasive cancer. A minority of cases do not fall clearly into either group, and in these, the age of the patient and signs and symptoms are helpful.

In answer to the second part of the original question, it is our experience that attempting to correlate an exact cytological pattern with the exact differentiation of an invasive carcinoma is extremely difficult. One can say, however, that when fibers are seen on smears of a proven in situ lesion, that the histological diagnosis is unequivocal.

THOMAS R. SIMON
Omaha, Nebraska, U.S.A.

To answer this question we reviewed cervical scraping smears and punch biopsies from 50 cases of invasive carcinoma and from 40 cases of carcinoma in situ of the cervix, selected only because of their availability and because the smears in each case had been diagnosed positive for malignant cells.

The smears were examined for the presence or absence of spindle cells.

The invasive carcinomas were classified histologically according to the classification of Martzloff as modified by McCormick and Belovich into three groups: (1) spinal-cell type, (2) transitional type, and (3) small-cell type. The spinal-cell type shows a high degree of keratinization, has no small cell component, and corresponds roughly to Broder's Grade I. The transitional type shows varying degrees of keratinization, is made up of both large and small cells, and corresponds to Broder's Grade IV.

An attempt to classify the cases of carcinoma in situ histologically was abandoned as impractical.

Spindle cells were seen in 2 of 40 cases of carcinoma in situ and in 20 of 50 cases of invasive carcinoma. Therefore, although they are rather uncommonly found in in situ carcinoma, their presence in an individual smear is of little value in suggesting whether the carcinoma shedding them is in situ or invasive.

Spindle cells were present in the smears from one of 15 cases of invasive carcinoma of small-cell type. Their site of origin in this case could not be determined from the biopsy material. They were present in the smears of all 6 of the invasive carcinomas of spinal-cell type and in 13 of 29 of those of transitional type.

The finding of spindle cells in smears from an invasive carcinoma then strongly suggests that the tumor of origin is of one of the more differentiated types, either spinal-cell or transitional.

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PETER STOLL
Heidelberg, Germany

The formation of spindle cells in the squamous epithelium and in the squamous carcinoma is a sign of maturity. I believe that the cytoplasm of benign and malignant cells undergoes the same cytochemical changes. The nucleus, however, will mature only in the benign cells and will become pyknotic, whereas the nucleus of the malignant cells retains the properties of the malignant nucleus: It will show mitotic division and will shrink with chromatin condensation only in cases of extreme maturation.

In the malignant cases in which spindle-shaped cells occur, one is justified in assuming that the squamous carcinoma has a tendency towards monocellular keratinization (cornification) and formation of horn pearls. Other parts of the same lesion may remain immature. The occurrence of spindle-shaped squamoid cells in the smear means, therefore, only that parts of the lesion exhibit maturation. It gives however, no definite information as to the general degree of maturation of the carcinoma.

DISCUSSION

FRIEDRICH BAJARDI, Graz, Austria:

The papers read by the three main speakers left no doubt that spindle cells found in smears were generally to be regarded as a symptom of maturity in epithelial tissues. To estimate the overall degree of maturity on purely cytological data seems impossible.

Moreover, it must not be forgotten that so-called spindle cell carcinomas, which might be described as immature tumors closely related to basal cell carcinomas, may equally be the source of spindle cells found in smears. Their origin can, however, be determined by cyanophilic cytoplasm.

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

We have compared 73 carcinomas in situ with 73 invasive carcinomas by means of histology and cytology.

a) Histology:

In 60 of the 73 carcinomas in situ we found a layer of spindle cells with spindle-shaped nuclei. The cells represented the uppermost layer of the unharmed surface, were PAS positive and had prekeratin formation, which has been recognized histochemically. They thereby suggested a stratum corneum. This layer is easily injured and is missing if a diagnostic curettage has taken place previously, or if for cytological purposes the surface has been scraped off too strongly, or if during the operation an alteration of the tissues with instruments or swabs has taken place. The lack of this layer can also be caused by causes other than artifacts. First, there are epithelial changes, which consist only of polymorph cells, without a "redifferentiation" (by Albertini - 1947) that would have permitted the formation of a basal cell layer as well as the maturation to plasma-rich and spindle-shaped superficial cells. Secondly, the upper cell layer can push away, especially

with severe subepithelial inflammation, or not even be formed. We found the surface cell layer consisting of spindle cells 28 times in 73 cases of invasive carcinoma. Obviously these changes, per se, have an inclination for severe ulceration and shedding away of the uppermost cell layer.

The following comparative findings were made:

	Carcinoma in situ	Invasive carcinoma
1. All epithelial layers a) basal cell layer b) polymorph, plasma-poor layer c) polymorph, plasma-rich layer, PAS positive d) spindle cell surface layer	21	3
2. Only the b, c, and d layers	35	24
3. Only the b and d layers	1	0
4. In addition to the layers 1 to 4, also a 5th layer: parakeratotic layer (clinically: leukoplakia)	2	1
5. Only layers c and d	1	0

In the 73 invasive carcinomas 14 pearl formations were found. In carcinomas in situ pearl formations are never found, and only in one core representing a glandular involvement could we find the first elements of a pearl of concentrically arranged spindle-shaped cells. In contrast to that, one could find twice as many superficial prekeratin containing spindle cells in carcinomas in situ (61:28).

b) Cytology:

The above preinvasive and invasive carcinomas were also examined cytologically. The procedure follows:

The absence of a distinct cellular form was only recorded if such elements were absent in all smears (usually 6-10 smears were examined). Spindle-shaped cells were found:

	In carcinoma in situ	In invasive carcinoma
1. Without prekeratin formation and with vesicular nucleus	25	20
2. With prekeratin formation and usually with pyknotic nuclei	23	31

SUMMARY

In histological sections carcinomas in situ are approximately twice as often covered by a superficial layer consisting of spindle-shaped cells than are invasive carcinomas (60:31).

In cytological smears spindle-shaped cells are found in carcinoma in situ and invasive carcinoma with about the same frequency (without prekeratin formation (25:20), with prekeratin formation (23:31).

I believe that spindle cells are pathognomonic for an irreversible malignant proliferation of the squamous epithelium and that their occurrence has an important practical diagnostic value in differential diagnoses of adenocarcinomas.

If only highly differentiated dyskaryotic cells are observed in addition to spindle cells, a carcinoma in situ may be expected. If only dedifferentiated tumor cells are found in addition to spindle cells, an invasive squamous epithelial carcinoma may be expected. A conclusive cytological diagnosis regarding the tumor type, however, should not be made since, for example, an invasive carcinoma may be surrounded by carcinoma in situ and the cytologist can not be certain where

the smear comes from and whether the physician has taken the cytological sample from all areas of the lesion.

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JEAN de BRUX, Paris, France:

I am almost in complete agreement with Peter Stoll. However, I would like to add this:

a) I have never seen any mitotic divisions in malignant spindle cells. I believe that the shrinkage of the nucleus and the condensation of the chromatin are constant and occur at the same time as the cytoplasmic modifications. Malignant spindle cells undergo the fate of normal epidermoid cells, but they retain the abnormalities of cancerous cells, even when they become mature.

b) The presence of abundant malignant spindle cells in a smear from a cervical carcinoma is always—in my experience—significant of deep invasion.

JORGE CAMPOS R. de C., Lima, Peru:

The presence of spindle cells is very suggestive of infiltrating carcinoma of a well-differentiated histological type. However, in an individual smear the presence of these cells alone is not enough to decide whether the carcinoma is in situ or invasive, and for this reason the total cytological picture must be taken into consideration, a picture which differs greatly in the case of cancer in situ and in infiltrating cancer. The description of these two pictures made by Siegler agrees with our experience.

RUTH M. GRAHAM, Buffalo, New York, U.S.A.:

To Dr. Siegler:

I have interpreted the question asked in the title rather differently than Siegler. The types of carcinoma of the cervix means to me squamous, adeno, or undifferentiated carcinoma. Siegler's discussion answers another question: "Is it possible to distinguish between invasive squamous carcinoma and in situ squamous carcinoma by the presence or absence of spindle-shaped squamoid cells?" Our own findings are similar to those of Dr. Siegler's. Fiber cells appear much more frequently in invasive squamous carcinoma than in in situ squamous carcinoma. Although this is true on a percentage basis it is of very little help in the individual case since in situ cases may have such cells and they may be completely absent in invasive squamous carcinoma.

To come back to the original question, I feel that the presence of fibers or spindle cells is pathognomonic for squamous carcinoma. If such cells are seen in the smear our report will read: "Positive, consistent with squamous carcinoma."

Are spindle cells a distinct type of maturation? Certainly they are morphologically. They have a cytoplasm which stains in many instances as the normal superficial cells, which is in marked contrast to the undifferentiated cell with little or no cytoplasm and no evidence of cellular maturity. Boschann points out in his section on the cyto-chemistry of these cells that they contain true keratin. This must mean a distinct type of cellular maturation.

To Dr. Simon:

Simon has interpreted the question as whether or not the presence of spindle cells correlate with the histologic grade of the tumor. We did such a study several years ago (1) and found no such correlation as Simon indicates. In one hundred and fifty two cases of invasive squamous carcinoma of the cervix the presence of fiber cells was compared with the histologic grade of the tumor. The tumors were graded according to Broder's classification. There was very little correlation. In 65% of the Grade II tumors there were fiber or spindle cells in the smear, in Grade III - 74% and in Grade IV - 66%. This difference of opinion may be explained partly by the fact that unfortunately in our series 74% of the cases were classified as Grade III histologically. In addition the histologic grade was taken from the routine hospital record and does not represent a careful histologic study. However, in our experience the fiber cell is a common constituent of the malignant cell population of most squamous carcinomas of the cervix.

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EMMERICH VON HAAM, Columbus, Ohio, U.S.A.:

Siegler's accuracy in making a cytological differentiation between invasive carcinoma and in situ cancers has never been matched by us, and we did observe large tumors in animals who completely failed to show any spindle-shaped squamoid cells. I agree with him that the attempt to correlate the cytological pattern with the exact type of invasive carcinoma is extremely difficult in most instances. Simon as well as Stoll accept the appearance of spindle-shaped cells as evidence that the tumor is one of the most differentiated types with a tendency toward keratinization and the formation of horn pearls. I would like to refer to Figure 13 of our paper on "Experimental Carcinoma of the Uterine Cervix of the Mouse" (1) where spindle cells appeared as elongated cells with scant, pale, eosinophilic cytoplasm and typical malignant nuclei without any evidence of keratinization and pearl formation.

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LEOPOLD G. KOSS, New York, New York, U.S.A.:

Perhaps my understanding of the question is slightly different from the other contributors. However, while I agree with everything that was said on the subject, I should like to emphasize that the presence of spindle squamoid cells is not necessarily associated with the presence of cancer. Spindle cells originate from the surface of abnormally maturing squamous epithelium and therefore their presence may be also noted whenever there is a tendency toward abnormal keratinization. As Stoll has mentioned, the nuclei of the benign spindle cells do not display, as a rule, the abnormalities that we have learned to associate with cancer. Especially in post-menopausal women with marked cervicitis, the presence of benign spindle cells is not infrequent and should be interpreted with caution.

JULIETA C. de LAGUNA, Mexico, D.F., Mexico:

Our results are similar to those presented by Siegler. In 1955, an evaluation of our material concerning the point of differential diagnosis between carcinoma in situ and invasive carcinoma showed that in 37 cases cytologically diagnosed as carcinoma in situ, biopsy study found 28 to be histological carcinoma in situ (76%) and 9 cases which, besides having areas of intraepithelial growth, had small zones of invasiveness. In 48 cases diagnosed cytologically as carcinoma in situ with areas of invasion, the histological study corroborated such a diagnosis in 37 cases (77%) and in the remaining 11 only areas of carcinoma in situ could be detected. In the last three years we have been able to study about 100 more cases achieving an 85% correlation between our cytological diagnoses and the histological ones.

Concerning the aspects related to the fiber cell, I would like to state that, for us, these cells, when typical, are malignant and characteristic of a highly differentiated squamous cell carcinoma. When they predominate in the smears and, furthermore, the rest of malignant cells show no big nuclear monstrosities, we are confident that in a very high proportion of cases most of the tumor mass corresponds to a Broders II growth.

LUBA A. TURNBULL, Montreal, Canada:

In my opinion, when referring to the spindle-shaped cancer cells, we must always remember the following three characteristics:

1. that these types of cells are always seen in invasive or clinical cancer of the cervix, where the deep epithelial cells are involved in a tumor.

2. that true spindle-shaped cancer cells are mature, highly differentiated cells.

3. that these types of cells are always seen in squamous carcinoma and never in adenocarcinoma.

In interepithelial carcinoma, I have sometimes seen elongated, oval nuclear forms in cancer cells. These cells usually appear singly in the smear, and I would simply call them cancer cells and not really spindle-shaped cancer cells.

CLOSING REMARKS

EDWARD E. SIEGLER, Cleveland, Ohio, U.S.A.:

In reference to some of the above comments, I would like to begin by saying that to me classical fibers or spindle cells mean carcinoma, and more specifically, squamous cell carcinoma, either invasive or in situ in type. To me, fibers usually mean invasive squamous carcinoma, but like any rule of thumb, no money-back guarantees accompany the report.

Much to my surprise, contrary to much of the literature, there did not seem to be any strong disbelievers in the cytologists' ability to differentiate in situ from invasive cervical cancer in most cases.

Regarding maturation, almost everybody except von Haam and myself, believes that the presence of spindle cells means a well-differentiated or highly differentiated squamous cell carcinoma. If one were to use Martzloff's classification, a well-differentiated tumor would be his spindle cell type which is a relatively uncommon histological grade (15% of his series). Yet, fibers are found much more frequently (70% in our series). This means to me that one cannot correlate this information with the usual histopathological punch biopsies of invasive cervical cancer, since the vast majority of these, at least on H & E sections are poorly differentiated, i.e., Martzloff's transitional cell type.

I do not know why there is so much interest in whether a spindle cell is a well-differentiated tumor cell since far more prognostic importance is placed on the clinical staging or Graham's SR than on the morphological type.

THOMAS R. SIMON, Omaha, Nebraska, U.S.A.: No comment.

PETER STOLL, Heidelberg, Germany: No comment.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

ARE SPINDLE-SHAPED SQUAMOID CELLS DERIVED FROM THE SURFACE OF THE LESION?

HERBERT K. FIDLER
Vancouver, British Columbia, Canada

Since spindle cells are found among freely exfoliated cells or in surface scrapings of cervical lesions, one would naturally assume that these cells are derived from the surface.

This assumption is confirmed by finding spindle-shaped cells situated on the surface in histological sections. Figure 1 depicts an intraepithelial squamous carcinoma of the cervix, the section being taken in the region of the external os. In this plane of section the surface cells are spindle-shaped. Since these cells are seen in cross section, however, one cannot be certain that some or many of them would not appear as flat squames in a smear; but the fact remains that no other cells in this lesion contain nuclei that are greatly elongated, thin and pyknotic: features that characterize the spindle type cell. Moreover, examination of the cone biopsy by step serial section revealed no other cells that would fit the description of spindle squamoid cells found in the direct cervical smears of this case. Thus one can conclude that the spindle cells came from this surface lesion. It is our experience during the examination of some 300 cone biopsies that spindle cells in the smear can be explained by these surface cells.

Identical cells are found in the deep gland extensions of intraepithelial carcinoma. In these situations, however, due to their deep location, they are less frequently shed and are probably rarely found in cervical smears. Almost without exception they are found in the layer most remote from the basement membrane and presumably represent obsolescent malignant cells.

An unusual way in which spindle cells may form is shown in Figure 2, a section from deep gland extension in intraepithelial carcinoma of the cervix. Elongated, pyknotic nuclei are seen in the parabasal layer and they stream outward through fields of more viable tumor cells to the center of the narrowed gland lumen.

Finally, spindle cells occur in deeply invasive carcinoma and are situated in the central, more mature part of the invading nidus (Figure 3). Because of their deep situation they are probably infrequently seen in cervical smears.

HERBERT LAX
West-Berlin, Germany

The histologist observes the cells in "section;" the cytologist observes the cells in the "plain."

1. Spindle-shaped cells with narrow, elongated, hyperchromatic nuclei which sometimes lie in multiple layers are found in the histological sections of normal, benign surface epithelium, mostly as a result of mechanic expansion (e.g. in cervical hypertrophy). As long as the surface epithelial tissue remains intact, these cells become more and more flat. However, if the cellular union is loosened, partially or completely, the tension is discontinued, and the cells resume more or less their original form. Fig. 1 shows a histological section with seemingly spindle-like cells which are, however, cytologically not "spindle cells."

2. Similar changes are observed in the incipient carcinoma in situ. The still normal, benign surface layers are being displaced and stretched by the markedly increased cellular propagation and increase in the volume of the nuclei which originate in the basal layers. Histologically, one also observes in these cases cells with a seemingly spindle-like shape which cytologically are not "spindle cells."

3. Spindle-shaped cells are found almost always in histological sections of well-preserved surface epithelial tissue of invasive and non-invasive carcinoma of every type of maturity. Fig. 3 shows an immature carcinoma with only one layer of flat, spindle-shaped cells. Figs. 4 and 5 are from a papillary, "spinocellular" carcinoma and Figs. 6 and 7 from a mature surface carcinoma. Figs. 4 to 7 also show free-lying, shed off "spindle cells" of various sizes and forms. All cells derive from the superficial layers.

The histologist cannot decide whether or not the spindle-like shape is artificially produced by sectioning or if the cells would retain their spindle shape in the cytological smear also. One can only consider as proven that a spindle cell is a special cell form which occurs in carcinomatous lesions.

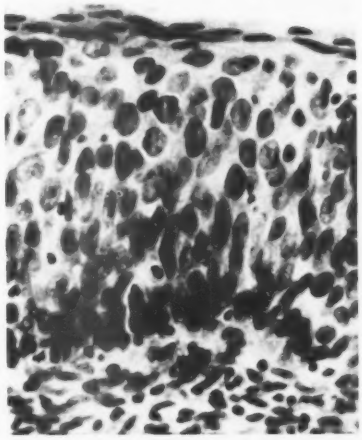

Fig. 1. Intra-epithelial carcinoma of the cervix the surface of which is covered by several layers of spindle shaped squamoid cells. X500. (HERBERT FIDLER)

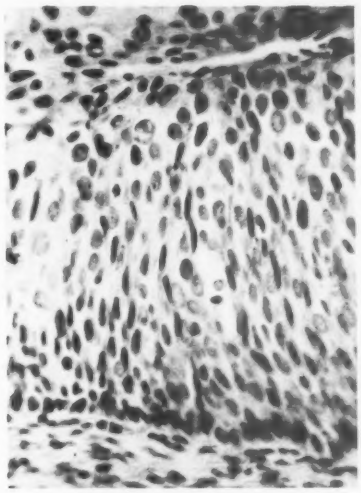

Fig. 2. Spindle cells developing in parabasal layer and streaming to the surface. X500. (HERBERT FIDLER)

Fig. 3. Carcinoma of cervix, deeply invasive in region of rectum, showing spindle cells in the central part of tumor nidus. X500. (HERBERT FIDLER)

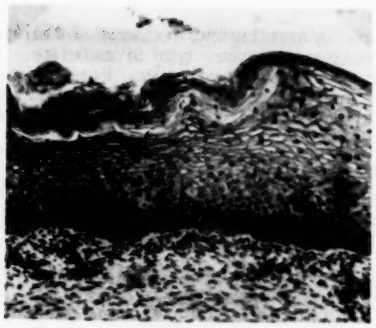

Fig. 1

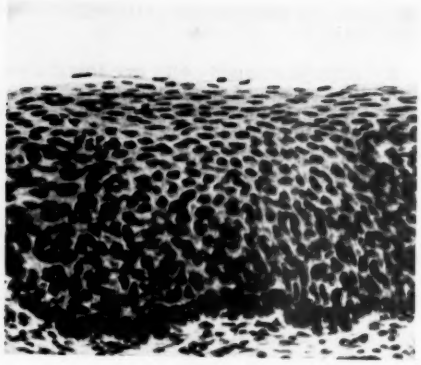

Fig. 2

Fig. 3

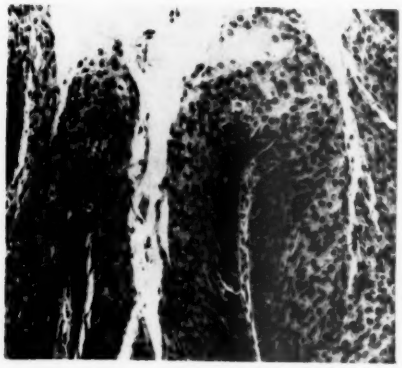

Fig. 4

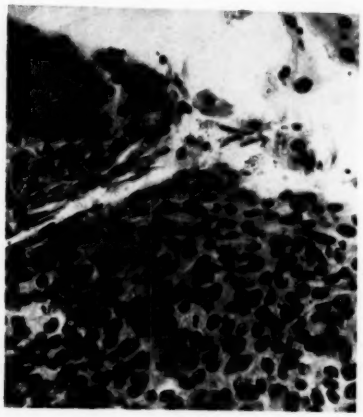

Fig. 5

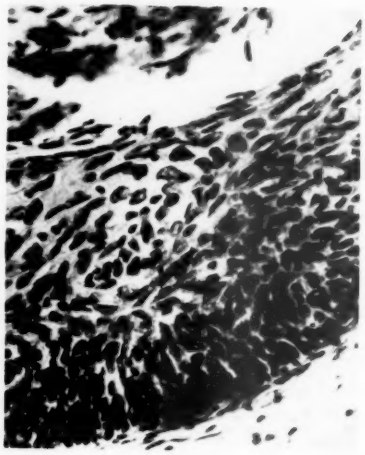

Fig. 6

EDMUND SCHÜLLER
Wien, Austria

It is my experience that the true, i.e., the "malignant," spindle-shaped squamoid cells derive from differentiated epidermoid carcinoma and practically always from invasive carcinoma. One can find these cornified or cornifying cells in the histological section in more or less markedly expressed pearl formations. The tumor cells exhibit here an appearance of onion skins. In cornified cancers these cells surround an amorphous keratinized mass. In the carcinoma which is not that well differentiated, these layered clusters are not as well defined and do not yet contain any keratinized center. Usually the spindle-shaped squamoid cells exhibit an eosinophilic cytoplasm, sometimes even a gold-orange cytoplasm. Therefore, they contain keratohyalin or keratin. The rather rare cyanophilic "malignant" spindle-shaped squamoid cells derive from solid carcinomas which exhibit only a certain tendency towards cornification, but which have to be included in the group of differentiated epidermoid carcinoma.

The classical spindle-shaped squamoid cells do not derive, in my opinion, from the surface of the carcinomatous squamoid layer, but from the previously described areas from within the carcinoma. These areas reach the surface either because the covering layers were already shed (due to exophytic tumor formation or to ulcerative tendency of the tumor) or because cells were scraped from the deeper layer of the tumor by means of the instrument when preparing the specimen.

There are the following diagnostic conclusions when evaluating the presence of spindle-shaped squamoid cells in the smears. One is justified in speaking of the existence of a differentiated epidermoid carcinoma, most probably an invasive one. Theoretically one might also consider the presence of endometrial adenocanthoma. In these cases, which are in my experience extremely rare, one would be brought on the right track by the detection of the intercurrent presence of "cancer" cells of the glandular type, which should be present in those cases.

DISCUSSION

EMMERICH VON HAAM, Columbus, Ohio, U.S.A.:

I fully agree with Lax that histologically many cells may assume the shape of spindle cells which are not spindle cells at all after exfoliation. This is particularly true in some of the fast-growing carcinomas where cells appear compressed and therefore assume the shape of spindle cells. The true fiber cells, as illustrated in my essay in the same number of the Acta Cytologica in Figures 1, 2 and 4, are so grotesque, however, that they never can be recognized in histological sections in their true shape. I agree with Fidler that those cells most likely represent obsolescent malignant cells, although they are not necessarily derived only from the surface of the lesion.

CLOSING REMARKS

HERBERT K. FIDLER, Vancouver, Canada: No comments.

HERBERT LAX, West-Berlin, Germany: No comments.

EDMUND SCHÜLLER, Vienna, Austria: No comments.

Symposium B

ADVANTAGES AND DISADVANTAGES OF MICROSCOPIC TECHNIQUES FOR EXFOLIATIVE CYTOLOGY AND OF STAINING TECHNIQUES OTHER THAN THE ORIGINAL PAPANICOLAOU METHOD

ADVANTAGES AND DISADVANTAGES OF THE MAY-GRÜNWALD GIEMSA PROCEDURE IN EXFOLIATIVE CYTOLOGY

PAUL LOPES CARDOZO
Leyden, Holland

I. GENERAL CONSIDERATIONS

In 1902 Giemsa (1) first described a blood film stain for malaria in which he used a combination of eosin and azure I. His definitive publication about this method appeared in 1904 (2).

Since then, far the best studied field in cytology, viz. hemocytology, has been based on studies with the M. G. G. -stain (or less satisfactorily with Wright stain, because the methylene azure B component in this technique does not come to its full effect).

Here one encounters not only all variants of malignant processes (considering for instance the many variations of myeloid leukemia and metastatic tumors (3) and multiple myeloma), but also many variants of benign processes: shift to the left, toxic granulation, Döhle bodies, vacuolization, aneosinophilia and eosinophilia (this last also in sputum, stomach sediment and pleural fluid), benign familial anomalies such as Pelger-Huet's, polychromasia, reticulocytosis, Heinz bodies, spherocytosis, target cells, megaloblasts, L. E. cells, among many others. In addition, the cytologist en passant can often give bacterial information, which is not to be wondered at since Giemsa originally published his method in the Centralblatt f. Bakteriologie as a stain for the malaria parasite. Staphylococci, Streptococci, Filaria, Plasmodium malariae and other parasites, such as appear in every hematological atlas, can be seen; but also meningococci and actinomycosis can be recognized.

The Giemsa solution contains methyl alcohol, glycerine and water as solvents and eosin, methylene-blue and azure B as dyes. These are, at least, the components of the Dutch brand I use, which is very constant in its composition. There is still a certain degree of mystery about what the precise original recipe of Giemsa was; he never published it, but like Meinicke, made it a manufacturer's secret. This is not of particular importance at the present, however, since various well-known European companies can deliver a satisfactory Giemsa solution.

If we consider the three components, it is the methylene-blue dye, especially Löffler's methylene-blue, in alcoholic solution, which gives a particularly good staining reaction. One can, thanks to the notable transparency, directly differentiate nucleoli, nuclear structure and the cytoplasm, in which even keratinization can be identified. Eosin, which is the most important part of the Hematoxylin-eosin and also plays a large role in the Papanicolaou stain, is actually a rather poor dyestuff. It colors no essential part of the cell. It is in fact the "background music" of the M(ay)-G(rünwald)-G(iemsa) stain. Methylene-blue and eosin together in methyl alcohol make up the components of the May-Grünwald solution.

Whenever one uses the Pappenheim method (4), that is May-Grünwald solution and then Giemsa solution, one increases the methylene-blue and eosin factors in the whole preparation. This is useful in blood and bone marrow staining, because the granulation of the neutrophiles becomes clearly visible.

The most essential point of the Giemsa stain is, however, that it is a good form of (acid) methylene-azure B coloring--the main dye of Romanowsky's combination. It remains somewhat uncertain what precisely one is coloring, but it will probably be possible in the near future to de-

termine this more exactly. At the moment it is already clear that, contrary to the situation with eosin, a very important differentiation can be made between D. N. A., which is purple-red stained in the nucleus, and R. N. A., which is blue (6, 7).

The Feulgen and the Pyroxine stains are, then, for the person well experienced in Giemsa staining, not essential.

The color difference of the cytoplasm, blue (basophilic) and rose (oxydophilic), is also useful insofar as the question of whether a cell is still young and acid (basophil) or already older and more alkaline (thus, oxydophil) is concerned. We are thus coloring vital parts of the cell with Giemsa stain, in contrast to Hematoxylin-eosin and related stains.

Occasionally one sees polychromasia, a mixture of red and blue tints, as with ciliated epithelium in which the red tint is usually unipolarly present where the cilia are or were. With degeneration and difficulties of identification this can sometimes be of help. Occasionally the mixture of colors gives special effects, as for instance "rain cloud-like" vaguely bordered cytoplasm of the epitheloid cell in tuberculosis, a staining effect that is approached by no other method.

Also, finding thrombopoiesis in a cell is an additional but sometimes useful help in differentiating megakaryocytes from Reed-Sternberg giant cells, especially in the spleen and bone marrow.

Of practical importance is the blue color often taken on by the nucleoli, which by its intensity is also an argument for malignancy. The special staining for sex chromatin is likewise unnecessary (8). Due to the richness in details, according to my belief, the oxydase coloring also is of little clinical importance now. I practically never use it with leukemia any more.

The transparency of the Giemsa stain, when correctly done, can be considered quite adequate. Sometimes one can increase this transparency by narrowing the diaphragm. Also, using Shell microscope oil or other paraffin substance to cover the preparation will be useful, especially for frequent changing magnifications of 100x and 500x (as I usually do). This last, frequent use of the oil immersion, meaning going into more detail, together with the fact that Giemsa coloring affords more details and colors vital parts of the cell, is the main difference in working methods. This also is a main reason that we, in those subjects in which "Giemsa-cytologists" have been able to concentrate, have "developed" a more sensitive and more detailed cytology, which seems to be more reliable and more informative, especially in needle-aspiration-cytology.

The consequence is that histology is no longer the golden standard for us but simply another currency where a constant rate of exchange exists, but where not always the same products are for sale. In other words, we have just written an article that shows that the results obtained with M. G. G. -cytology in the diagnosis of Hodgkin's disease, especially with lymph node aspiration and also with bone marrow and other material, is more exact due to the fact that we can distinguish all sorts of details in the Reed-Sternberg cell and its precursors which the histologists are not able to see (9). Such a standpoint will not surprise the true hematologists among the readers. Have we not learned through our daily experience to be convinced of the superiority of the cytological principle (Giemsa) in diagnosis of multiple myeloma (10), leukemia, infectious mononucleosis, pernicious anemia, and other hematological diseases? Although one does color both the nucleus and cytoplasm, the name "panoptic" coloring is still a bit of an exaggeration for the M. G. G. -staining. Very important components of the cell, namely mitochondria and Golgi apparatus, are not made visible. For reticulum fibrils (Gomori stain), phosphatase staining, the staining of glycogen, among other aldehyde-containing substances (Hotchkiss stain), other staining methods are necessary. Indeed, even for the simple reticulocyte still another dyestuff (brilliant cresyl blue) is required.

An important argument in favor of the Giemsa stain is the simplicity of the method: it requires little time, space or money. If the result is unsatisfactory, then there are few factors on which to check up. One does not have to check an entire row of solutions, but is concerned only with:

1. Methyl alcohol (fixation)
2. Buffer solution
3. Giemsa solution
4. The condition of the slides before making the smears
5. The measuring cylinders in which the stain solution is prepared
6. The coloring procedure

Of these, the first 3 are very easy to check or to replace, as necessary. The cleaning of the slides in absolute alcohol is not a problem. One must at most check if this has been done. For the measuring glasses and coloring dishes, one must insist on cleaning only with distilled water, never tap water. After being cleaned, the measuring glasses should be filled with distilled water until further use.

II. COLORING PROCEDURE

Certain general points about the coloring procedure must be understood first. The preparation is allowed to dry in air. Only then is the fixing performed. This so-called dry fixation is very easy in practical work, since this by-passes a complicated phase of the Hematoxylin-eosin staining and related methods (among others, Papanicolaou staining). Furthermore, the alcohol-ether fixation in itself causes some degree of shrinkage (11).

1. Fixation (in containers with methyl alcohol)

   Very fresh material (not more than 1 hour old) - fixation for 30 minutes
   Fresh material (1-3 hours old) - fixation for 15 minutes

   Preferably, we fix material that is 4-24 hours old for $\pm$7 minutes (it may be longer, but no more than 30 minutes).

2. Before beginning the staining one should rinse the slides with freshly prepared Giemsa solution (per 1 cc. phosphate buffer pH 6.8, two drops of Giemsa solution).

3. The staining time is variable. We estimate this according to the following:

   a. With great number of cells, longer staining time;
   b. With more blood present, longer staining;
   c. For few or degenerated cells (stomach contents) or non-fresh material, shorter staining period;
   d. The brand of Giemsa stain plays a role.

Staining Time Table for Orientation (2 drops Giemsa to 1 cc. buffer):

Sputum	8-10 minutes
Swab smears needle-aspiration	6-10 minutes
Dab preparations, rinse fluid	5- 8 minutes
Stomach contents of all sorts	3- 4 minutes
Urine sediment	2- 4 minutes
Cerebrospinal fluid	1- 1-1/2 minutes (1 drop Giemsa in 2 cc. buffer)

4. At the end of staining, one must control the result microscopically: restain if too light, decolorize if too dark by placing in re-distilled water for 20 minutes to 1 hour (preferably not in distilled water and never in tap water). Strongly overstained slides should be put 1-3 minutes in a dish containing methyl alcohol. By leaving them longer in methyl alcohol, one can achieve complete decolorization. For this reason, in principle, the Giemsa stain can never fail.

5. It is also important that the preparations be liberally rinsed in horizontal position in order to avoid stain precipitation. If the upper side is clean, one can rub off the lower side.

Quick-staining According to Pappenheim:

During an operation one can do a quick staining on air-dried smears as follows:

a. 2 minutes in methyl alcohol,
b. 1 minute in undiluted May-Grünwald solution,
c. 1 minute in 50% diluted May-Grünwald solution,
d. pour off,
e. add dropwise undiluted but filtered Giemsa solution from the filter,
f. gently rinse after 2-3 minutes.

Even quicker is the staining with Löffler's methylene-blue (1 min.), preferably after 2 minutes in methyl alcohol. With this one can usually see quickly whether there is malignancy or not, but one has a less detailed and less meaningful preparation than with Giemsa staining.

Very Slow Staining:

On the other hand, with strongly diluted Giemsa solution the staining time is greatly prolonged, but the nuclear staining is better and the sex chromatin, for example, can be seen. In an understaffed laboratory working at high tempo the schedules given above are a reasonable compromise.

III. SUMMARY

1. The quintescence of the May-Grünwald Giemsa stain is the methylene azure B component in the Giemsa solution. In our non-hematological cytological work we now omit the May-Grünwald solution. Its components, eosin and methylene-blue, are also present in the Giemsa solution itself, although the staining conditions are different.

2. The Giemsa technique stains essential parts of the nucleus, viz. D. N. A. and R. N. A. Blue cytoplasm means the cell is acid, hence the name basophilia. It nearly always means that the cell is young. The opposite, the pink stained (oxidophilic) cytoplasm, often means adult cells or at least cells with more alkaline cytoplasm. Polychromasia is a mixture of pink and blue staining. It is not merely a picturesque effect, but has several points of cytological importance. The best Giemsa-cytologist is the best "connoisseur" of the nuclear structures and of the variations of polychromasia and other modifications in staining properties in nucleoli and cytoplasm. These specific Giemsa variations also make it possible for the cytologist better to differentiate between neuroblastoma, Hodgkin's,disease, leukemia, etc., than the histologist. Moreover, many benign diseases, among them, tuberculosis, are within the routinely made diagnoses of the Giemsa cytology.

3. The best Giemsa stain--euphemistically called a "panoptic stain"--is at least the best "panoptic" stain, giving many more details than any other cytological stain (thrombopoiesis, granulation, etc.). Other important parts of the cell, especially the mitochondria and the Golgi apparatus, however, are not visible in this way.

4. The "dry fixation," the drying in air and fixation with methyl alcohol, is the easiest procedure available. It has, moreover, a great margin of safety; it is not dependent on exact timing. It also gives less shrinkage than the ether-alcohol procedure, which in turn is much better than the histological techniques.

5. Because of its simplicity the Giemsa technique is quick, easy, space-saving. Faults are easy to find because so few factors and relatively few stages play a role in the Giemsa stain.

6. Poorly stained Giemsa smears can be quickly decolorized in methyl alcohol and restained. The same is true even for very old smears. So far our (often uncovered) smears are still in perfect condition, after 10 and more years.

7. The Giemsa stain has a firm hematological background and, on the other hand, it brings hematological cytology, "needle aspiration cytology" and exfoliative cytology under one heading. In the first two headings everybody agrees the Giemsa stain is definitely superior to the Hematoxylin-eosin, and allied stains, e. g., the Papanicolaou stain. We feel it reasonable to recommend that each great medical center, also in the U. S. A., regard itself as incomplete in cytological respect as long as it has merely a laboratory in which only the exfoliative cytology is practiced, either using the Papanicolaou stain or the Giemsa stain or still other stains.

Every leading medical center should establish a second laboratory which uses the Giemsa or allied stain for needle aspiration cytology, a field as large as the exfoliative one.

8. Like the Papanicolaou stain the Giemsa stain is very useful for mass screening. Because of its quickness, cheapness and simplicity I even feel that it is the best technique yet available for this purpose, at least in cytology of the respiratory tract, and also in urological cytology and perhaps even gynecological cytology, a field in which it is not often used. In regard to the gastrointestinal tract, I have not yet come to a conclusion as to the usefulness of the Giemsa stain.

9. Because of the possibilities of progressive and regressive staining, correction and even total decolorization by immersion for a few minutes in methyl alcohol, any slide can be stained just as one wishes it.

10. The staining techniques for the Giemsa stain are given. The stain in its speed-modification is useful as an operation room technique, requiring about 5 minutes.

Even a bit quicker is the Löffler methylene-blue stain, which is, however, definitely inferior in the details of cytological information.

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The author wishes to thank Dr. James (Histologish Laboratorium te Amsterdam) for his aid in the preparation of this paper.

FRANTIŠEK LUKSCH
Prague, Czechoslovakia

In the year 1954 we replaced the then routinely used Papanicolaou technique for cancer screening in our laboratories by a modified Giemsa staining procedure. The procedure is as follows:

1. Fixation in methyl alcohol
2. Rinse in water
3. Stain in Giemsa solution (2 gtt. per ml.) for 20 minutes
4. Air drying and storage without a cover glass
5. Screening of the slides at various magnifications covering the material with immersion oil.

Our experience with this technique is based on studies of more than 9000 routine specimens. Comparative studies with nuclear staining techniques (hematoxylin, silver impregnation) and cytoplasmic or mixtures of stains, such as ones by Papanicolaou, Shorr, Magendie, Pasini and others, gave sufficiently good results, especially in regard to secondary regressive changes. The technique by Cramer and Stamm (1) does not offer, in my opinion, any advantage for the cytological detection of cancer.

The advantages and the disadvantages of the above described Giemsa modification for the cytological routine work may be listed as follows:

Advantages	Disadvantages
(1) Brief staining procedure (which is important in laboratories with shortages of personnel)	(1) Nuclear chromatin less distinct than with the Papanicolaou Technique
(2) Comparatively inexpensive technique	(2) Quantitative nuclear staining differences are less marked (nuclear hyperchromasia)
(3) Slides may be easily filed without cover glasses	(3) Lack of plasma reaction
(4) Excellent demonstration of microbiology, especially trichomonads	(4) The technique is not useful for hormonal evaluation
(5) Smears cannot be artificially overstained	

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DISCUSSION

J. de BRUX and J. DUPRÉ-FROMENT, Paris, France:

Compared, from the standpoint of execution, to the Giemsa or Pappenheim techniques, the staining method of Papanicolaou or that of Harris-Shorr might appear longer and more delicate; it must be performed in several stages, which must be times with extreme precision, rendering the retouching of a coloration nearly impossible. The chief difficulty lies in the fixation, which must be realized immediately, for otherwise the quality of the smear is permanently compromised. However, these are minor difficulties and simply require careful attention on the part of the personnel where intensive training is not available.

From a morphological standpoint, the Papanicolaou method is an excellent routine stain, as opposed to the panoptical techniques, which it would seem preferable to reserve for certain

isolated cases, such as the search for cytolyzed trichomonads. On a smear stained by the Papanicolaou method, the trichomonas appears as a bluish corpuscle of ill-defined form, the nucleus having the aspect of a shadowy ring; whereas, stained by the May-Grünwald Giemsa technique, the nucleus is clearly visible and of a reddish color which differentiates it easily from spots of mucus or from fragments of cytoplasm.

As has been remarked by Luksch, the May-Grünwald Giemsa staining method is practically never used in hormonology: the cytoplasm is pale, with eosinophilia and cyanophilia poorly differentiated, rendering the interpretation difficult with the lower magnifications. Moreover, a higher magnification is undesirable, for an overall view is necessary in order to determine the grouping of the cells, the degree of folding and the number of polynuclear cells. And yet, perhaps it is principally in this field that this method might be utilized, for in many other fields the pyknosis of the nucleus is the only valid hormonal criterion.

As far as the detection of malignant cells is concerned, the panoptical colorations prove to be much less exact than the Papanicolaou technique. The chromatin network is indistinctly outlined and, for better appreciation, requires examination under immersion. The examination of an entire slide at such a magnification is long, tiring and risks becoming a source of errors. Above all, it does not give the overall picture upon which, to a certain degree, the cytologist's conclusion depends.

What, in fact, are the morphological criteria which permit the differentiation of a normal malpighian or cylindrical cell from a metaplastic or malignant cell of the same origin?

These criteria include, especially, the nuclear characteristics; the study of the cytoplasm is more secondary, for all cells may undergo a cornification, whether they be benign, dysplastic or malignant; and all cells may present vacuoles, either in degenerescence or in secretory activity. In final count, the determinant element is a minute examination of the nucleus in its smallest details: its form; the ratio of nuclear-cytoplasmic material; the increase, if any, of the nuclear membrane; conformations and folds in the chromatin; the number and extent of nucleoli and, finally and especially, the chromatin: its dispersion in dust, in grains or in clumps, as well as its density; certain hyperplastic nuclei appearing "blown up," nearly emptied of their substance; other nuclei, of malignant nature, appearing on the contrary as a thick, compact and uniformly black chromatin matter.

In hematology, on the other hand--a discipline which, we may say, recognizes only one routine coloration, that of May-Grünwald Giemsa--the elements of interest are essentially the cytoplasm and the granulations, if any, in order to classify the cell in a determined rank and, within the rank, to establish its degree of maturation. Of course the character of the nucleus is far from negligible, since a parent, or so-called "blastic" cell is noted not only by the intense basophilia of its cytoplasm, but also by a high nuclear-cytoplasmic ratio and by the fineness and distinctness of its chromatin which, moreoever, is nucleolated. In like fashion, the leukemic cell, an abnormal blastic element, is characterized by the combined character of its grayish cytoplasm and its nucleolated, sometimes "bumpy," incised nucleus.

But, generally speaking, whereas the nucleus represents an important diagnostic element in hematology, the conclusion does not depend solely on its characters, but also on a whole group of other factors (cytoplasm, disappearance of other sanguine groups, leukemias, monomorphic or exclusive proliferation of only one group).

In cancer cytology, on the contrary, the diagnosis depends almost solely upon nuclear morphology, the other elements having a comparatively contingent role in reinforcing a hesitant diagnosis based on the nucleus alone. Hence, an extremely detailed study of the nucleus is indispensable, and for this the most expressive method of staining must be chosen.

P. Lopes Cardozo believes that the most expressive method is that of May-Grünwald Giemsa. In this case we are inclined to adopt it, if it can enable us to resolve the arduous problem of the borderline lesions, as well as the less difficult one of distinguishing the dysplastic from the malignant cell.

GUILLERMO TERZANO, Buenos Aires, Argentina:

Pundel wrote "the final value of a cytologist is less a question of the staining technique he uses than the pictorial memory he has collected by a long cytological experience." This remark sounds especially good as an introduction to the chapter "staining techniques."

Everybody knows that Pouchet made brilliant (it was 1847) observations with unstained vaginal smears, and everybody knows that any cytologist can obtain better results with a technique he is accustomed to, rather than with another one he is not familiar with. When I was asked to read slides stained with hematoxylin and eosin, May-Grünwald Giemsa, etc., I was as handicapped

as the pathologist, familiar with hematoxylin and eosin, whom I asked for his opinion on slides stained according to the Papanicolaou technique.

A technique may be considered as adequate for vaginal smears when it can give a correct staining of the nuclei, nuclear chromatin detail, and good differentiation between eosinophilic and cyanophilic cells.

Advantages such as "brief procedure" or "inexpensive" should not be a matter of concern (under normal circumstances) in hormonal diagnosis or cancer diagnosis.

The Papanicolaou technique is not an excessively expensive procedure, neither is it exceedingly long, and it is not so complicated as to have one look for easier procedures. The staining of 20 slides at a time makes the procedure easy enough.

Dry smears can be stained with May-Grünwald Giemsa and reported, but it is only once in a while that we have to face this circumstance, since doctors have agreed to send to the laboratory smears fixed in ether-alcohol or use Ayre's glycerin technique. Either one has always been possible.

CLOSING REMARKS

P. LOPES CARDOZO, Delft-Leyden, Holland:

Drs. de Brux and Dupré-Froment view the problem only from the standpoint of the gynecological exfoliative cytologists. I agree that for these the staining procedures based on hematoxylin, namely the Papanicolaou and Shorr techniques, are preferable at this time. The hematoxylin staining principle, however, proved insufficient for hemocytology and needle-aspiration cytology, and was considered inferior by no lesser a man than Paul Ehrlich.

The nuclear pattern was always considered extremely important, by exfoliative cytologists and hematologists alike. Did Pappenheim not already state: "The nucleus is the coat-of-arms of the cell."? This seems quite a different view than the one expressed by de Brux and Dupré-Froment on hemocytology, which I am afraid no true hematologist will share. However, even if the de Brux and Dupré-Froment discussion were only restricted to gynecological exfoliative cytology, I cannot imagine why they believe that the nuclear structure in cells stained properly according to the Giemsa technique can be difficult to see. In any case the criticism is quite without basis for the cytological examination of needle aspiration and dab-hemocytology. In conclusion I remain convinced that the Giemsa stain is much better than many cytologists yet believe.

FRANTIŠEK LUKSCH, Prague, Czechoslovakia:

Essentially, I agree with the opinion of de Brux and Terzano. The best argument against the usefulness of differences of opinion concerning staining procedures was the citation by Terzano of Pundel's motto in this issue.

I am sure attributes such as "brief procedure" or "inexpensive" should not be decisive in the selection of any methods. They may be of some importance, however, when two equivalent methods are in competition with each other. Unfortunately, they must be considered decisive where the equation "time equals money" holds true.

ADVANTAGES AND DISADVANTAGES OF THE HEMATOXYLIN-EOSIN PROCEDURE IN EXFOLIATIVE CYTOLOGY

HANS IGEL
Berlin, Germany

The use of a staining solution in histology as well as in cytology is based on empirical grounds. In spite of several studies, it has not been possible to clarify the nature of staining processes. The staining of cellular structure is dependent upon the speed of diffusion and concentration of the solution and upon the nature of the object (1, 2, 3). On the other hand, the electrical factors of the dye-substances and also of the substrate are important, i.e., the acid-alkali content of the solution in relation to pH of the cellular elements (4, 5, 6).

One of the important staining methods in histology is the hematoxylin-eosin method. After the Second World War we did not have the necessary staining solutions for the Papanicolaou method, so we used hematoxylin-eosin dye substances for our cytology work. After fixing the smears with alcohol and ether in the usual manner, we stained with Ehrlich's acid hematoxylin for 10 minutes and with 0.1% watery solution of eosin for one minute. The hematoxylin stains the nucleus blue-violet. The nucleoli and the cell granules are well demonstrated. The time of the staining must be exactly controlled as the structure of the nucleus, particularly, could not be recognized if overstained. A correction by simple washing is not possible; also, rinsing with 1% HCl solution does not help.

We gave up the staining of the cytological smears with eosin because the intensive red coloration of the erythrocytes was disturbing. Therefore, we then stained only with hematoxylin (7, 8). We have had good results with this method, although the cell structure is not as clear and transparent as with the Papanicolaou stain. The hematoxylin method can be used in cancer diagnosis, but it is not suitable for cyto-hormonal studies. An essential advantage is the shorter time for the staining procedure, as well as its economy. Therefore, the hematoxylin staining method is suitable for small hospitals where there is not enough personnel, and also for physicians in general practice.

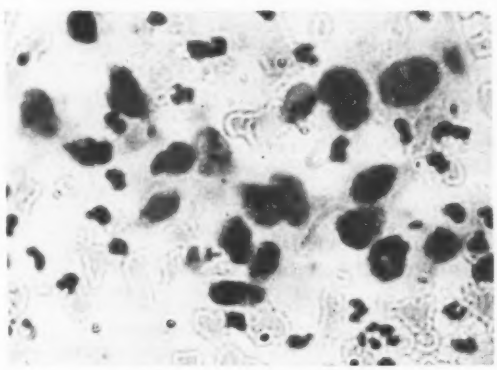

Fig. 1. Cancer cell complex stained with hematoxylin

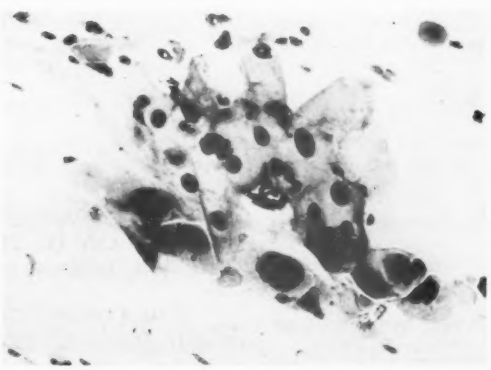

Fig. 2. Cancer cell complex stained with Papanicolaou method

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DISCUSSION

GUILLERMO TERZANO, Buenos Aires, Argentina:

As the author points out, monochromatic techniques, such as hematoxylin and eosin, do not give the required good transparency of the cytoplasm and do not demonstrate the difference between eosinophilic and cyanophilic cells.

Since good nuclear coloration can be obtained with hematoxylin and eosin, its use could be recommended for cytological cancer diagnosis, but since it is not histology (where the pathologist can see the architecture of the tissues) but cytology (where only the morphologic pattern of isolated cells leads to the correct evaluation), one must always have in mind the danger of an improper staining procedure. For hormonal diagnosis, hematoxylin and eosin might be useful for the quick evaluation of an atrophic smear (parabasal cells) or a normal smear (superficial-type cells).

CLOSING REMARKS

HANS IGEL, Berlin, Berlin: No comments.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

ADVANTAGES AND DISADVANTAGES OF THE SHORR PROCEDURE IN EXFOLIATIVE CYTOLOGY

J. PAUL PUNDEL
Luxembourg, Luxembourg

For routine cancer screening, the Papanicolaou and Shorr staining techniques are both convenient. But for hormonal diagnosis by vaginal smears, the Papanicolaou technique has given, in my experience, less satisfying results. In my laboratory it seemed most practical to have only one staining technique for both cancer and hormonal diagnosis, so I have adopted and used exclusively, for more than 10 years, the Shorr technique with differential stain S3. I prefer this technique for the following reasons:

1. For hormonal diagnosis, it is important that one be able to determine the Eosinophilic Index with a maximum of precision. The Papanicolaou technique gives a wonderfully colored picture, but the intermediate staining reactions between green or blue and red sometimes make it very difficult to establish the precise Eosinophilic Index. Control tests have shown that even the same cytologist can arrive at different Eosinophilic Indices when examining the same smear. Furthermore, the Papanicolaou technique is rather sensitive and even minor modifications in the different steps, the pH of the vaginal contents, the fixatives or the rinsing solutions of alcohol can produce artificial variations in the eosinophilia (1, 2, 3, 6, 7).

The Shorr technique, on the other hand, is less sensitive to such modifications in the different steps or in the pH, and gives only two basic staining reactions, green-blue and red, with a minimum of intermediate or bicolored cytoplasmic reactions. Comparisons in many cases have shown that the Eosinophilic Indices established by different cytologists for the same smear, presented only minor differences (less than 5%), while in the smears stained with the Papanicolaou technique, the differences sometimes were higher than 20%.

2. The Shorr technique is simpler and less time-consuming than the Papanicolaou procedure, and even important accidental variations in the staining times or the pH have no serious consequences. If the Papanicolaou technique is used and should give excellent results, it is very important that the variations are carefully respected.

3. The Shorr technique permits excellent study of the nuclear structure and has the great advantage in permitting a precise distinction between the nucleoli, which stain Burgundy red, and the sex chromatin or other chromatin condensations. This better visualization of the nucleolus can be very helpful in the diagnosis of cancer and of the genetic sex.

The disadvantages of the Shorr technique are:

1. The transparency of the cytoplasm is sometimes not as clear as with the Papanicolaou technique, especially if the Shorr is used for the staining of smears of origin other than the vagina or the uterus.

2. It is difficult to get a good commercial Shorr stain following the original S3 formula.

The simple S3 staining technique without hematoxylin has no practical value for routine examinations.

Personally, I prefer the Shorr technique, but this preference should be considered only as a personal opinion dependent upon the local and practical working conditions. The diagnostic accuracy of a cytological laboratory depends, indeed, for a great part, on the quality of the staining technique, but one should not forget that the final value of a cytologist is less a question of the staining technique he uses than of the pictorial memory he has collected by a long cytological experience.

To obtain this experience it is more important that he familiarize himself with one technique than try to find out which of several rather similar, equally good techniques would be the best for every cytologist.

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CLARICE DO AMARAL FERREIRA

Rio de Janeiro, Brazil

Many cytologists have discussed Shorr's staining procedure and told me it is no good for nuclear staining and that they prefer to use Harris hematoxylin. I think they are really referring to the single differential stain, the third modification (S3) of Shorr, that we do not like either. The first modification of Shorr to the Masson and Papanicolaou methods includes hematoxylin.

In 1940, E. Shorr (1) thinking the Papanicolaou staining procedure insufficient for cytoplasmic detail, tried to modify the trichromic method of Masson, especially for demonstrating cornification. His first modification (S1) is as follows: a) fixation of the smear while still wet in alcohol-ether, hydration before staining, since he used water soluble stains; b) nuclear staining with Harris hematoxylin, a mixture of Ponceau xilidina, Acid fuchsin and Orange G for the karyopyknotic, eosinophilic, superficial cells and erythrocytes; and Bright green for the intermediate cells. Before the Bright green, the smear is passed through a 3% phosphotungstic acid. Dehydration is necessary before mounting the smear.

The same year Shorr modified the method (S2) (2) a little, and substituted for the Ponceau plus Acid fuchsin plus Orange G, Biebrich scarlet plus Orange G plus acetic acid; he also changed the mordant to phosphotungstic acid plus phosphomolybdic acid and the Bright green to Fast green (stain from Nat. Anil. Chem. Co.).

In 1941 Shorr tried a single differential stain (3) putting into a single stain Biebrich scarlet, Orange, G, Fast green, phosphomolybdic and phosphotungstic acids, all in an alcohol-acetic acid solution. The Shorr stain method has passed through many other modifications, such as the ones by Fuller (4), by Majendie, Zozol and Pautrizel (5), Isaac and Wurch (6), etc.

In 1942, when we started practicing cytology in the Gynecological Clinic, Victor Rodriquez (7) started to try out the Shorr stain for his thesis on ovarian function. It was a good stain; we became accustomed to it especially because we could obtain the dyes more easily than the Papanicolaou stains. We have also experimented with many other staining methods, including that of Gomori (8), using Shorr's stain for hormonal evaluation, and Papanicolaou's for cancer diagnosis.

Comparing the Papanicolaou theory methods, we have the impression that Papanicolaou's staining procedure gives more nuclear transparence, and therefore less cell differentiation than we want for hormonal evaluation. With the Shorr staining procedure, differentiation between eosinophilic superficial and cyanophilic superficial or intermediate cells permits excellent smear evaluation. From the cancer diagnosis viewpoint, we need a good nuclear staining. Both methods stain the nuclei by hematoxylin, and in both the chromatin is sufficiently well visualized to permit a correct diagnosis. We once tried the single differential stain of Shorr and obtained bad results with it, so have never used it again. Taking care to obtain a good, mature hematoxylin, to change the other stains periodically, and to provide a good washing of the smears with water after each stain (except the green), we have obtained, for routine purposes, very satisfying slides with S1 and S2, in which the height of proliferation can be evaluated and the nuclei satisfactorily studied for deviations from normality.

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DISCUSSION

JORGE CAMPOS R. de C., Lima, Peru:

In order to evaluate the advantages and disadvantages of the staining techniques of Papanicolaou and Shorr, one must first establish what he is looking for and what he hopes to stain by the particular technique. I believe that when one deals with heterogeneous samples, vaginal, uterine, sputum, bronchial washing, gastric washing, etc., and the main object is the diagnosis of cancer, then the Papanicolaou method should be the method of choice. On the other hand, if the laboratory deals mainly with hormonal studies on vaginal cytology, then the Shorr staining technique is most advantageous.

There is another factor that one should bear in mind: in small laboratories with few facilities and few qualified personnel, the Shorr staining technique has the advantage of being simpler and faster.

I agree with Pundel, however, that the important thing is the experience which the cytologist gains while working with one special technique.

PIERRE HAOUR, Lyon, France:

I had the pleasure to see Pundel's slides in his laboratory and I agree that the staining procedure he uses is excellent for nuclear structures as well as for cytoplasmic differentiation.

However, I don't think I will give up using the Papanicolaou staining technique in cancer diagnosis, even if the hematoxylin-Shorr seems simpler and gives similar results. We tried the technique proposed by Pundel, but then we returned to the Papanicolaou staining procedure, with the HCl differentiation.

Of course it is advisable that a standard technique be adopted by all cytologists. But in cancer diagnosis, when one has been accustomed for years to one technique, it seems rather impossible to change it, without a prejudice to one's own experience in cytology.

CAMILLE LICHTFUS, Strasbourg, France:

Besides our use of the combined stain of hematoxylin with zinc sulfate (Papamiltiades) and the original S-3 stain of Shorr for all our gynecological cytology, for hormonal as well as cancer cytology we wish to emphasize the use of these stains in a particular field: in more than 50 genetic determinations of sex by means of the vaginal and buccal smear, we have been able to show evidence each time of the chromatin of Barr when female genetic sex is involved, and that without any difficulty.

The coloration is very interesting in the cases where the buccal smear alone is useful: premature infant (4 cases), absence of vagina, etc. And we do not have to resort to other staining methods. Unfortunately, some of our colleagues in France have shown the difficulties of getting the original Shorr stain.

HORST SMOLKA and PETER DOERFFLER, Kiel, Germany:

We agree with both the Main Speakers that in the Shorr staining technique the exclusive use of the stain S-3 is not satisfying in the cytologic diagnosis and that the combination with hematoxylin should be the method of preference for most purposes.

The cytoplasm generally shows a greater contrast in color between red and green-blue if the Shorr staining technique has been used, so that the exclusive determination of the Eosinophilic Index may be easier. This plays a certain part especially for less trained and less experienced persons. As far as the doubtlessly more sensitive Papanicolaou staining method is concerned, the intermediate staining reactions, i.e. reddish transitional shades, are often referred to. In our experience particularly the polychromatic stain EA-31, which was much used in Germany some years ago, showed these transitional colors, which we only rarely observe now when applying EA-50. The brilliancy of color and the differentiation of the shades in the Papanicolaou procedure also depend to a large degree on the frequent renewal of the staining and rinsing solution! Modifications and shortened procedures of the Papanicolaou technique which were occasionally recommended always proved inferior to the original method in our experience.

However, in the Shorr staining technique less transparency of the cytoplasm as well as a generally darker staining with less delicate structures of the nuclei have to be put up with. Consequently, occasional errors in the establishment of the Karyopyknotic Index can be less easily excluded. This plays a still more important part in the hormonal control than an Eosinophilic Index which possibly has not been precisely determined, as the nuclear pyknosis presents a more sensitive criterion for estrogenic activity. In cancer diagnosis the darker staining of the nuclei may furthermore lead more easily to falsely doubtful or falsely positive results.

After parallel examinations of about 2000 smears—both these methods have been carried out on the same material for the purpose of comparison—we have arrived at the conclusion that the Shorr staining technique is very useful indeed in the hormonal control, but that, with a view to the totality of diagnostic possibilities in the cytologic field (inflammation, carcinoma, etc.) it proves inferior to the Papanicolaou staining method.

GUILLERMO TERZANO, Buenos Aires, Argentina:

I would like to say that according to my experience, both Papanicolaou's and Shorr's techniques are convenient for qualitative hormonal diagnoses by the smear method, but for cancer diagnoses I did not find a better stain that the one of Papanicolaou.

For one reason or another other techniques have been used in our laboratory. No one (including Harris-Shorr, Shorr S-1) has given the satisfactory results we get with Papanicolaou's original technique.

Sometimes we have not observed good differentiation between eosinophilic and cyanophilic cells, but that was when the staining solutions were exhausted (old, over-used, etc.). False eosinophilia (in those cases in which the Eosinophilic Index is not yet higher than the Pyknotic Index), as may happen when Trichomonas vaginalis is present, partially in dried smears, etc. was easier to detect when smears were stained according to the Papanicolaou technique; and I feel more secure using this technique to classify cells, with evaluation based upon folding edges, curling edges, etc.

Since we are only able to talk of tinctorial affinities of the vaginal cells treated with a known determinate solution, it becomes apparent that if we consider how the cells take the dyes, we should consider also how the cytologist likes it. But at the present time, provided that doctors use fresh ether-alcohol solution and fresh staining solutions, Papanicolaou's technique is the most adequate one, simple and inexpensive enough to continue using until a specific staining technique is developed.

Pundel and Ferreira have described so clearly the procedure, the advantages and disadvantages of the Shorr staining technique, and Pundel's personal comments are so good, that apart from congratulating the authors, there is little to say in discussing these papers.

I would like to add that when I have to discuss Papanicolaou's technique I feel it is a kind of "disadvantage" to have been at Papanicolaou's laboratory and have tried during many years to follow his work as closely as possible. But I must make clear that it is not that I am attached to Doctor "Pap." It is that since 1946 Papanicolaou's technique has been "my" technique because it is the best I have found.

CLOSING REMARKS

J. PAUL PUNDEL, Luxembourg, Luxembourg:

In my paper I have presented my own experience with the Shorr technique, which is limited strictly to vaginal, cervical and endometrial cytology. I agree completely with the discussants concerning the respective advantages and disadvantages of the Papanicolaou and Shorr techniques. If samples from elsewhere than the vagina or uterus have to be examined, I believe, also, that the Papanicolaou technique, with its modifications, should be chosen. If I continue to use the hematoxylin-Shorr technique for gynecological cytology, the reason is that originally when stains were difficult to obtain in Europe I had many difficulties with the available Papanicolaou stains. Therefore, I tried the Shorr technique. If I now obtain excellent results with this technique, it is because the assistants and technicians in my laboratory have thoroughly studied all the possibilities and disadvantages of the Shorr technique. Now we can claim to show an excellent technique. However, I must confess that this point was reached with difficulty. Many things had to be corrected before we were able to perfect our present technique, which is quite simple, but only for the cytologist or technician who knows all the tricks of the technique.

The purpose of my paper was not to claim that the hematoxylin-Shorr technique should become the technique of choice, but only to present my personal experience. I repeat that for good cytological work the choice of technique is less important than experience and results (which alone should be the criterion for a technique). If one cytologist has had experience and obtains excellent

results with either the Papanicolaou or with the Shorr technique, I cannot see why he should change his technique for another, unless it is to obtain an international standard technique. In the future, if it appears to be necessary to use only one standardized technique, and if the Academy should propose for this the Papanicolaou technique, I would accept the decision of the Academy.

CLARICE DO AMARAL FERREIRA, Rio de Janeiro, Brazil:

I agree with Pundel, as referred to by Jorge Campos, that the important thing is the experience which the cytologist has gained while working with one special technique. This is important since when examining a slide, after some years of practice, we "feel" that a given cell is atypical. Perhaps, our subconscious knows better cytology than does our conscious mind. To distinguish the minimal atypia, it is important to always use the same method. However, I think that providing a good stain for nuclear chromatin is the main factor, especially for cancer diagnosis.

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THE COMMENTS WILL BE PUBLISHED
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ADVANTAGES AND DISADVANTAGES OF THE MODIFIED PAPANICOLAOU TECHNIQUES IN EXFOLIATIVE CYTOLOGY

CONSTANTIN HEROVICI
Villejuif, Seine, France

By the action of acetic acid on Bismarck brown, one can obtain a nuclear acid stain, marronine. When mixed with Biebrich red, Brilliant green, Aniline blue and Orange G, this provides a polychromatic stain in one single procedure suitable for cytological specimens.

Preparation of the Mixed Stains (Solution I. G. R. #347)

1. Nuclear Stain:

Fifty cc. of pure acetic acid are heated in a vessel almost to boiling point. In this 1 g. of Bismarck brown is dissolved, and 50 cc. of distilled water are added immediately. From this mother solution, which can be kept a long time, 10 cc. are taken and allowed to boil slowly until evaporation is complete.

The powder is placed in a drying oven at 56°C and allowed to stay there for at least 24 hours.

By this method, Bismarck brown is changed to marronine, the name that we have given to this new nuclear stain. It has acquired slightly acid properties and is miscible with other dyes in the final solution. It is kept in a 1% alcoholic solution.

2. The basic solution is prepared in the following manner:

0.35 g. of Biebrich red (R.A.L.) and 0.25 g. of phosphotungstic acid are dissolved in 10 cc. of warm distilled water; 10 cc. of absolute alcohol are then added to this solution.

0.45 g. of Brilliant green and 0.04 g. of Cotton blue are dissolved in 10 cc. of warm distilled water. Immediately 10 cc. of absolute alcohol are added.

0.20 g. of Orange (R.A.L.) and 0.25 g. of phosphomolybdic acid are dissolved in 10 cc. of warm distilled water. The orange hue of the mixture should deepen until a maroon color is obtained; one then immediately adds 10 cc. of absolute alcohol.

The three solutions are mixed together when cool.

The final solution is obtained by adding this basic solution, very slowly, almost drop by drop, to 10 cc. of the 1% alcoholic solution of marronine and 10 cc. of distilled water, taking care to avoid precipitation. The solution is made up to 100 cc. by adding 20 cc. of 50% alcohol. To this is added 1 cc. of acetic acid, and it is allowed to mature for 24 hours. The solution is filtered and is then ready for use.

Marronine	0.15 g.
Phosphotungstic Biebrich red	0.35 g.
Brilliant green	0.15 g.
Cotton blue	0.04 g.

Phosphomolybdic Orange G	0.20 g.
Acetic acid	1 cc.
50% alcohol	to 100 cc.

Technique

1. Fix in alcohol-ether
2. Wash in distilled water
3. 50% alcohol in acetic acid (1%) 30 seconds
4. Stain 3 minutes
5. Wash rapidly in 50% alcohol
6. Differentiate and dehydrate in the alcohols – 70%, 80%, 95%, 100%, xylol
7. Mount with Canada balsam

Results

Eosinophilic cells	red
Cyanophilic cells	blue
Nucleus	
chromatin	maroon
nucleolus	red
Nuclear membrane	blue
Blood	orange

When the stain is getting old, one may decolorize in Lugol's solution, 1 minute, after immersion in the stain, taking care to remove the excess Lugol's solution in the alcoholic immersions. One can include a rapid washing in distilled water between the dye and the Lugol's solution.

Advantages of this Method

1. The short time taken for this method permits few mistakes due to handling.

2. The use of marronine never causes an overstaining of nuclei, and its property of specifically staining the chromatin allows nuclei which are rich in chromatin to be differentiated from those that are poor.

The co-existence of marronine and Biebrich red permits selective staining, differentiating the chromatin from the nucleolus, which appears bright red.

As we already know that chromatin is composed of desoxyribonucleic acid and the nucleolus of ribonucleic acid, this technique is of cytochemical value in differentiating the two acids and seems particularly suited to cytological control in irradiation.

Disadvantages of the Method

In vaginal smears which contain an abundance of red blood cells, the nuclei containing little chromatin are much less visible than if they were stained with techniques using hematoxylin.

Summary

The author describes a mixture of five histological stains suitable for cyto-diagnosis. The nuclear stain in this mixture is a new chemical entity derived from Bismarck brown, called marronine. Cytoplasmic stains are: Biebrich red, Brilliant green, Cotton blue and Orange G, with phosphotungstic and phosphomolybdic acids as mordants. To stain in a single procedure has the advantage of selectively differentiating the chromatin from the nucleolus: the former staining maroon, and the latter, red.

NO DISCUSSION

ADVANTAGES AND DISADVANTAGES OF THE SUPRAVITAL STAINING TECHNIQUES IN EXFOLIATIVE CYTOLOGY

WARREN R. LANG
Philadelphia, Pennsylvania, U.S.A.

Supravital staining techniques have not had wide application in gynecological cytology, most investigators preferring the fixed, stained smear. We have used the technique of Rakoff (1), however, both in office and clinic practice for over five years and have found it valuable mainly in determining the approximate state of ovarian function and the occurrence of ovulation. It is also of value in assessing the presence of infection and bleeding. The technique is relatively simple and can be carried out in a few minutes.

Smears are taken, preferably from the lateral vaginal wall, in a patient who has not douched for at least 24 hours. These may be obtained either with a pipette or a cotton-tipped applicator. The secretions thus obtained are placed in 2.5 cc. of physiologic saline solution to which is added 3 drops of:

10% aqueous solution of Light Green	83 ml.
1% aqueous solution of Eosin Y	17 ml.

The solution is then gently agitated and a drop or two placed on a glass slide. A cover slip should be used so the individual cells are more easily visualized.

The smear is then examined under low and high dry magnification, the staining being roughly comparable to that obtained with the Papanicolaou smear. Superficial karyopyknotic squamous cells (eosinophilic with Papanicolaou technique) stain pink, intermediate cells, blue-green. Nuclear definition is not very satisfactory. By subsequently adding a few drops of Lugol's solution to the test tube, glycogen-containing cells can also be determined.

The advantages of the stain are the rapidity and ease with which it can be used. The major advantage over the routine wet smear with saline solution is the differentiation between intermediate and superficial cells. The main disadvantages are the instability of the stain (the solution must be prepared weekly), the fact that the preparation is not permanent and the absence of definitive nuclear staining.

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DISCUSSION

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

I agree with Lang in all points. The technique reported by Rakoff offers good results for an immediate diagnosis, e.g., in sterility cases. Lang points out that the demonstration of the nuclei is not sufficient. This is why we would prefer phase microscopy as an immediate diagnostic procedure. With this technique one can also obtain, without any additional staining, immediate information as to the presence of the following:

1. superficial, intermediate and parabasal cells.
2. pyknotic or vesicular nuclei.
3. normal or abnormal nuclei.
4. normal or suspicious cytoplasmic structure.
5. trichomonads.

Both the supravital staining procedure as well as the phase contrast technique have the same disadvantage, namely that the specimens cannot be preserved, and therefore, not be reread. Immediate photography is

1. difficult, since unfixed cells which are suspended in a fluid medium usually move, due to the molecular movement of the fluid medium and due to the marginal negative pressure, etc. The photographs are, therefore, often not clear enough.

2. more expensive and more time consuming than the preparation of a fixed and stained permanent smear.

3. insufficient because one can demonstrate photographically only a small portion of the smear.

It is also necessary for hormonal evaluations by means of the smears to compare the present findings with previous smears in order to determine changes in the hormonal condition. This is important, e.g., in order to determine whether or not a cycle is anovulatory, whether or not there is an uterine amenorrhea with intact ovarian function, whether or not one deals with a secondary amenorrhea which is due to early pregnancy or to glandular cystic hyperplasia in follicular persistency or due to ovarian failure, and finally, whether or not the hormonal condition during pregnancy is changed, or a threatened abortion is hormonally influenced.

Institutions with efficient cytological laboratories will, therefore, prefer fixation and staining. Practicing physicians and institutions which do not have the possibility to cooperate with a cytology laboratory will prefer supravital staining procedures and phase microscopy. Cytological training centers will, however, employ all known techniques, even if it might be only for the purpose of teaching and research. I believe that I am in full agreement with the main speaker in this conclusion.

CLOSING REMARKS

WARREN R. LANG, Philadelphia, Pennsylvania, U.S.A.:

I thank Boschann for his comments. As stated, we have found the Rakoff stain extremely helpful and easy to utilize and to interpret. Our own experience with phase contrast microscopy is only beginning. However it does furnish much more nuclear detail and is therefore of value as a carcinoma screening technique. As with supravital methods it also has the disadvantage of lack of permanency.

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ADVANTAGES AND DISADVANTAGES OF THE KARYOLOGIC TECHNIQUE IN EXFOLIATIVE CYTOLOGY

LUIGI CUSMANO
Novara, Italy

The microscopic structure of chromatin is very stable in environmental variations and changes only in cancer cells; in benign tumors, in functional hyperplastic tissues and in acute and chronic inflammations, no changes are detectable.

The specific structural changes of the chromatin are recognizable particularly in the resting cells, because chromosome abnormalities in cancer cells during mitosis are not always present.

The squash method is considered the best to study the nuclear structure. The material, fixed in acetic alcohol (2/3 70% alcohol : 1/3 acetic acid) and stained with aceto-carmine and aceto-hematoxylin, is squashed between slide and cover glass, so as to obtain the dissolution of the cytoplasm and to set the nuclei in a single mononuclear layer. The preparation is then dehydrated and mounted. When it is necessary to shorten the time of preparation, it is possible to study the fresh (nondehydrated) slide directly.

In the nuclei of non-malignant resting cells, the chromosomes are seen as chromatin threads, among them, some of particular thickness (heteropyknotic), which behave as organizers of the nucleolus (usually two per nucleus in human cells) (Fig. 1), while in malignant neoplastic cells, the resting nuclei are either richer in chromocenters (more than four) and all chromatin is strongly stainable (Fig. 2), or they are visible as very pale filaments with no chromocenters, together with very big and numerous nucleoli.

The latter observations were made for the first time by Caspersson and Santesson (1), who called the two kinds of cells "A" and "B" types, respectively.

These characteristics of the resting nuclei (which involve shape, number, size and aspect, either of the chromocenters or of the nucleoli) are considered as an index of malignancy.

It is very easy to employ this technique in the case of cancer of the female genital tract, taking three samples of vaginal material, i.e., a) spontaneously exfoliated material from the vagina, from the uterus portio and from the endometrium, b) artificially exfoliated material from the cervix uteri, and c) after intravenous injection of an ecbolic substance which, producing a valid contraction of the myometrium, permits the expulsion of the fluid from the uterine cavity into the vagina.

A positive or a doubtful answer, although it does not claim to prove in a definite way the presence, or, even less, the localization of the cancer, gives the possibility of proving the presence of cells, the chromatin of which shows structural modifications of neoplastic type. Only then all other means should be employed to check with more precision both the place and the degree of development of what has been indicated by the karyological investigation.

The agreement between karyological and histological examinations in symptomatic tumors reaches a percentage of 90.4, while, in asymptomatic tumors, there is 99.3% agreement. This is explained by the fact that the presence of diffuse necrotic processes in invading cancers is very often responsible for false negative diagnoses. More complete statistical data are available in the following monograph: "La Cariologia Nella Diagnosi Delle Neoplasie" (2).

This ambulatory examination, which may be easily used on a large scale, as compared to the cytologic one, offers the advantage of a simple technique and of an easier reading and

interpretation of the preparations. Furthermore, while having the same diagnostic possibilities as the cytologic examination for the diagnosis of the malpighian carcinoma, at both the infiltrating and the ulcerative stages, it proves to be a much more sensitive method for the detection of precancerous lesions and of the preclinical cancer, where the study of cytoplasm is of very slight diagnostic value.

Finally, this technique is remarkably favorable when the investigation involves haematic material, because the cytoplasm dissolving reagents dissolve the haematic mass also.

The karyological investigation is, therefore, worthy to be employed, not particularly in cancers with definite clinical symptoms, but in "silent" cancers, i.e., without evidence of any clinical symptoms of malignancy. The only disadvantage in comparison with the cytological method exists in the fact that the cytological method permits us to establish, with fair approximation, the place of origin of the exfoliated cells.

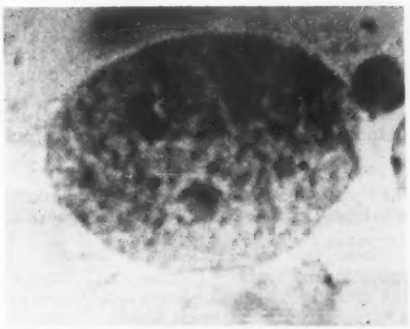

Fig. 1. Normal epithelial nucleus (Cusmano)

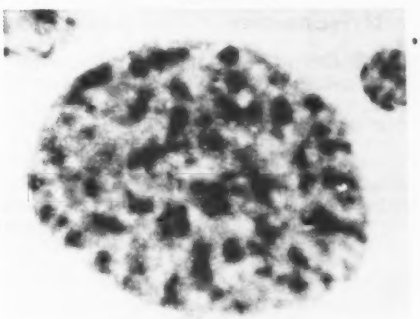

Fig. 2. A - nucleus (Cusmano)

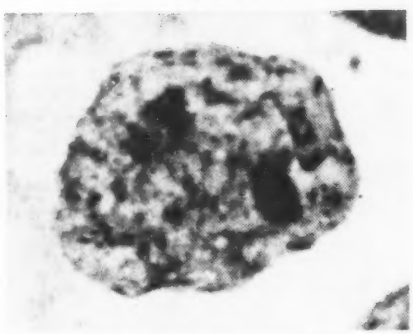

Fig. 3. B - nucleus (Cusmano)

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DISCUSSION

CLARICE do AMARAL FERRIERA, Rio de Janeiro, D.F., Brazil:

Since learning of Cusmano's and Caspersson-Santesson's work, I have carefully directed my attention to the importance of the nucleus in the early diagnosis of cancer. This is only a contribution from our experience.

Since last January, we have included in the routine examination at the Dispensary of Gynecology the examination of fresh material stained by 1 percent solution of acetic toluidine blue as a first cytological screening (1). The material from the cervix is placed on a clean glass slide in a drop of saline solution, stained with a drop of the toluidine and covered. The chromatin stains clearly and permits a diagnosis of atypia as easily as in a fixed-stained smear. In the first 300 cases, it was possible to correlate the findings in the malignant cases with smears stained by the Shorr technique. In two cases in which blood did not permit the correct diagnosis in the fixed smears, a diagnosis of cancer was possible with the toluidine blue stain. We found also that the diagnosis of the endocervical cells was easier using fresh material stained with toluidine blue than with Shorr's. The presence of blood does not disturb the cellular picture in the former because only the nuclei stain clearly.

Another topic I wish to speak about is a study on sex chromatin of dyskaryotic cells (on smears stained by the Shorr technique), and I would like cytologists to check to see if my first observation is correct:

I counted the sex chromatin in 25 cases (7 cases of intraepithelial cancer and 18 cases of cervicitis and dysplasia) in which cytologic study showed malignant and/or dyskaryotic cells. The percentage of nuclei with sex chromatin in the normal and dyskaryotic nuclei is as follows:

In the non-malignant cases, I found visible sex chromatin in 12-45% of the normal nuclei, with the average at 25%. In dyskaryotic cells, sex chromatin was visible in 12-56% of the cells, with the average at 26%. The difference between the two percentages can be seen in Table I.

In the cancer cases, I found a wide variation in the number of normal nuclei with sex chromatin—from 2 to 44%, with an average of 15.5%—and in atypical nuclei, sex chromatin was present in nuclei from 3 to 22%, in an average of 6%. I wish to point out that from the 7 cases of cancer only one had a high percentage of sex chromatin in the nuclei; in the others, it was low (Fig. 2).

It is possible that the failure to see the sex chromatin in dyskaryotic cells of malignant smears is due to the great masses of chromatin; however, it may be a fact to be considered in the evaluation of dyskaryotic cells in the differential diagnosis of a benign or malignant case.

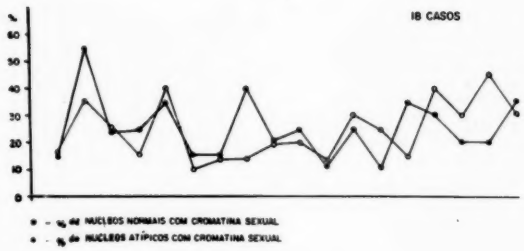

Fig. 1

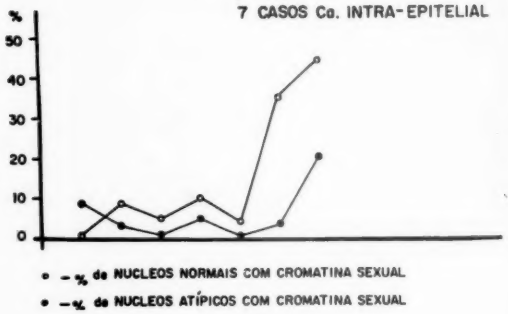

Fig. 2

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HANS KLAUS ZINSER, Cologne, Germany:

The technique of the karyological diagnosis is an example that an intensively practiced modification of an original method may lead to excellent results in the hands of a group of researchers. I was very much impressed by the authors' results which were published in their monograph entitled "La Cariologia nella Diagnosi della Neoplasie." Stimulated by the previous publications by Cusmano, we have tested this technique and compared it in simultaneous studies on stained smears according to the Papanicolaou technique. However, we did not succeed in finding any advantage in the karyological examination of the smears. The observations of the nuclear system which were inaugurated by the reports of Caspersson and Santesson do not seem to me to add any significant criteria which might refine the practical diagnosis. As a matter of fact, we have found by experience that the routine cytological technique is superior to the karyological technique in the detection of early malignant lesions when dealing with dyskaryotic cells. Dyskaryotic cells are better distinguished if one can evaluate the cell form in addition to the nuclear properties. These critical remarks, however, are not meant to diminish the theoretical value of the karyological examinations, which certainly have widened our knowledge of the cancerization of the cell.

CLOSING REMARKS

LUIGI CUSMANO, Novara, Italy:

To Dr. Ferreira:

I do not disagree because the observation made in the discussion that "the presence of blood does not disturb the cellular picture" has also been expressed by me since my early works. However, I am not in a position to give my point of view concerning sex chromatin as I have never applied myself to this subject.

To Dr. Zinser:

From a doctrinal viewpoint, the concept remains that the specific cytologic modifications for the affirmation of a neoplastic picture belong to the nucleus.

The possibility, therefore, of evaluating the phenomenon in an absolute sense and not in a relative manner, as with the Papanicolaou cytologic technique, represents, if not an end point, at least a starting point to investigate further the innermost problem of cancerogenesis.

For instance, the concept of dyskaryosis is inadmissible in a karyologic sense because the karyologic evaluation in a positive sense stands exclusively on absolute and not relative data, which on the contrary is required by the Papanicolaou cytologic evaluation.

As for the advantages or disadvantages from a technical viewpoint, only after employing for a long time the two methods contemporaneously is it possible to form judgment regarding the results obtained on equal material.

Undoubtedly both methods, in experienced hands, give approximately equal results. But the results obtained with the Papanicolaou cytologic method certainly involves more difficult labor and more time in reading the preparation; in less patent cases the method can give a higher quantity of false negatives. The karyologic method, giving easier and swifter reading, runs the risk of a higher quantity of false positives, which, however, does not betray the purpose of this method, because both methods need histological findings in order to reach a final conclusion. Considering this, the approach is easier through a positive finding, even false, than through a negative one.

ADVANTAGES AND DISADVANTAGES OF THE PHASEMICROSCOPIC TECHNIQUE IN EXFOLIATIVE CYTOLOGY

GEORGE L. WIED
Chicago, Illinois, U.S.A.

For routine diagnostic cytological examination, phasemicroscopy may be applied in the following situations:

1) on unfixed and unstained specimens, replacing the routine Papanicolaou technique;

2) on unfixed and unstained specimens, as a prescreening technique to eliminate completely normal smears and to decide whether or not an additional smear will have to be fixed and stained routinely;

3) on unfixed and unstained specimens as a routine office technique in addition to the routinely performed Papanicolaou smears for immediate cytohormonal and microbiological classification and determination of inflammatory reaction;

4) on fixed and stained specimens for photomicrography;

5) on fixed and stained specimens for standardization of karyopyknosis.

The advantages and disadvantages of this technique are discussed under separate headings.

I. On Unfixed and Unstained Specimens Instead of the Routine Papanicolaou Technique

In 1951 a comparative evaluation of phasemicroscopy and the Papanicolaou method as a routine cytological technique was reported (1): unfixed and unstained specimens from 1850 patients were examined under the phasemicroscope; the same patients also had specimens fixed and stained according to the Papanicolaou method. The results of this comparative study show that substituting a routine examination of unfixed and unstained material under the phasemicroscope for the routine Papanicolaou cytological examination was an impractical, inadequate and inefficient modification. After seven years the author feels that the statements made in this paper are still fully valid.

The disadvantages are:

a) The slides cannot be permanently filed, since they are practically useless if dried, unless one stains the air dried smears.

b) The unfixed specimens cannot be mailed for outside evaluation or consultation.

c) The preparation of the specimens requires special care: very thin material, very thin cover glasses, covering the margin of the cover glass with fat or other protective materials if smears are to be kept useful for at least a few hours.

d) The cells in the specimens are mobile. This is especially unfortunate for consultation of the cytologist by his technicians because very often the cells in question cannot be found again.

e) The change of the magnifications requires a time-consuming readjustment of the condenser in some makes of microscopes.

f) The use of the oil immersion objective brings the objective into direct contact with the cover glass of the specimen, causing the cells to float away.

g) The presence or absence of hyperchromasia cannot be determined.

h) Bloody specimens which may be evaluated if fixed and stained are usually useless because of the poor contrast in fresh bloody specimens.

i) Immediate photomicrography is the only way of recording cellular features.

j) A complete interpretation of the unfixed and unstained specimen requires extensive training. A cytologist who is an expert on fixed and stained slides might find himself completely at a loss in attempting a final reading on unfixed material.

k) Phasemicroscopic examination of unfixed slides is more tiresome for the eyes than examining fixed and stained specimens.

l) The diagnostic accuracy is not higher, but usually lower than with the routine Papanicolaou technique.

On the other hand, the only advantage of the technique is seeing the cells without artificial changes due to fixation and staining. However, I cannot find anything of diagnostic importance in this fact, especially if one considers that our entire histological diagnostic work is based on artifacts.

Summarizing this particular application of phasemicroscopy: examination of unfixed and unstained material as a replacement for the routine Papanicolaou technique offers only disadvantages without any significant advantage.

II. On Unfixed and Unstained Specimens as a Prescreening Technique to Decide Which Smears Have to Be Fixed and Stained

With some experience the cytologically interested clinician can decide which smears are "entirely normal" and which smears are "not entirely normal" by examining the unfixed and unstained specimens under the phasemicroscope (2). Many gynecologists already examine fresh drops of vaginal fluid for Trichomonas vaginalis. Using the phasemicroscope for detecting Trichomonas (3) is not only easier but at the same time permits differentiation of cellular details, which is not possible with the normal bright field microscope. If the gynecologist has had cytological training, he can probably eliminate a fair percentage of the "entirely normal" specimens, thereby channeling only the remainder of the slides to the cytologist for interpretation after fixation and staining.

The two systems for office prescreening are:

TABLE I. TECHNIQUE OF OFFICE EXAMINATION OF FRESH SPECIMEN UNDER PHASE-CONTRAST MICROSCOPE

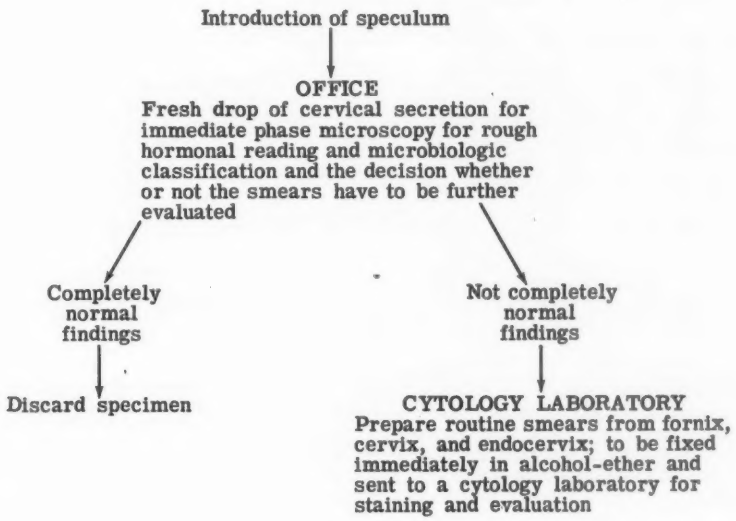
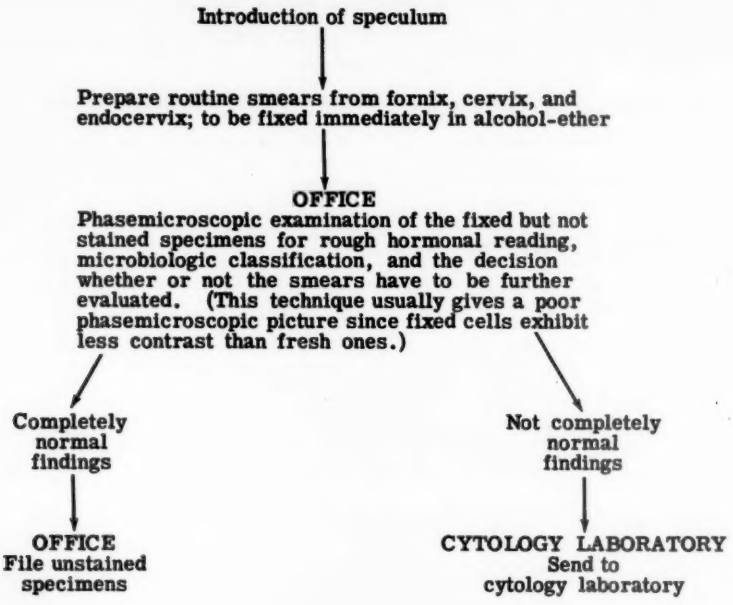

Introduction of speculum
OFFICE
Fresh drop of cervical secretion for immediate phase microscopy for rough hormonal reading and microbiologic classification and the decision whether or not the smears have to be further evaluated
Completely normal findings
Not completely normal findings
Discard specimen
CYTOLOGY LABORATORY
Prepare routine smears from fornix, cervix, and endocervix; to be fixed immediately in alcohol-ether and sent to a cytology laboratory for staining and evaluation

TABLE II. ALTERNATIVE OFFICE TECHNIQUE


Introduction of speculum
Prepare routine smears from fornix, cervix, and endocervix; to be fixed immediately in alcohol-ether
OFFICE
Phasemicroscopic examination of the fixed but not stained specimens for rough hormonal reading, microbiologic classification, and the decision whether or not the smears have to be further evaluated. (This technique usually gives a poor phasemicroscopic picture since fixed cells exhibit less contrast than fresh ones.)
Completely normal findings
Not completely normal findings
OFFICE
File unstained specimens
CYTOLOGY LABORATORY
Send to cytology laboratory

The first system requires more training on the part of the gynecologist since the "entirely normal" slides are discarded. The second system has the disadvantage that the fixed slides are more difficult to evaluate than the unfixed ones.

The disadvantages of applying phasemicroscopy as a prescreening technique are apparent:

a) The clinician must have a phasemicroscope (rather expensive), in addition to a minimum cytological training on unfixed material.

b) The technique falls short of the ideal, that of being able to give every woman the benefit of an annual cytological examination by means of a fixed and stained specimen examined by an experienced cytologist.

c) The elimination of a fair percentage of the smears by the prescreening technique used by the clinician may result in a rare, but possible, oversight of a malignant lesion since not all smears will be checked by a cytologist. On the other hand, if one considers that in many laboratories technicians eliminate a considerable percentage of the specimens, which are probably never seen by the cytologist in charge, the above argument seems to be less valid.

The advantages of the technique are:

a) The patient can get an immediate report that her smear shows only "entirely normal" cells.

b) The clinician can determine, roughly, the microbiology of the vagina at this time and with more training, he can give a cytological evaluation of the hormonal condition while the patient is still in his office.

c) With some training the clinician can eliminate as "entirely normal" many of the slides, thus providing the scarce cytological laboratories with specimens which represent diagnostic problems only, rather than overloading them with a bulk of normal specimens.

Summarizing this particular application of phasemicroscopy, one can say that although it certainly does not represent an ideal solution, it is still the second best choice and definitely

superior to the many gynecological examinations performed without any cytological examination, which, I believe, still occur in the vast majority of cases at the present time.

III. On Unfixed and Unstained Specimens As an Additional Routine Office Technique

The only disadvantage of this particular application of phasemicroscopic examination is that the entire gynecological examination becomes more time-consuming.

The advantages are apparent: the microbiological classification and the hormonal analysis of the slides can be made immediately, while the patient is still present (4). This in itself takes care of a significant percentage of the diagnostic cases in the gynecological office practice.

The technique is applied as follows:

TABLE III. PHASE MICROSCOPY AS ADDITIONAL ROUTINE TECHNIQUE

Introduction of speculum

OFFICE	CYTOLOGY LABORATORY
Fresh drop of cervical secretion for immediate diagnostic and therapeutic decisions and decision when the patient has to return.	Simultaneously prepare routine smears from fornix, cervix, and endocervix; fix immediately in alcohol-ether and send to cytology laboratory for evaluation.

In addition to the immediate examination of the unfixed and unstained material, the gynecologist takes a routine smear for fixation and staining according to the Papanicolaou method. In teaching institutions the resident physician who sees the patient in the outpatient department and reads the fresh smear is also required to check his findings on the stained specimens later on in the cytology laboratory. This provides him with some knowledge of exfoliative cytology for his future practice and also illustrates methods of cooperation with a cytology laboratory.

IV. On Fixed and Stained Slides for Photomicrography

For photomicrography of cells the use of the phasemicroscope on fixed and stained material usually results in a considerably better picture, due to better contrast and the demonstration of more details with a phase-equipped microscope. Some types of phasemicroscopes exhibit a marked chromatic aberration, however, and are not suitable for this particular use of the instrument, especially if the photographs are taken with colored film. If phasemicroscopes are to be used for photomicrography, it is suggested that they be tested initially for photomicrography with a color film; if the colors are distorted the equipment will not be useful for this particular purpose. A powerful low voltage lamp is mandatory for photomicrography with the phasemicroscope.

V. On Fixed and Stained Slides for Objective Determination of Karyopyknosis

The determination of the Karyopyknotic Index is done for hormonal evaluation of patients or a relative assessment of the duration and efficiency of administered estrogens. The determination of karyopyknosis with a bright field microscope is dependent upon subjective factors (5). Karyometry cannot be used routinely in determining nuclear size (smaller than 5μ). The phasemicroscope offers here a unique, practical way of differentiating objectively and reproduceably between a dense nucleus (pyknotic) and a nucleus which permits some light to pass through it (vesicular). With a phasemicroscope the light is deviated by dense corpora and the dense corpora appear to shine a bright red color, whereas the non-pyknotic nuclei remain the same color as the stained nucleus (6). One can best make use of the phasemicroscopic determination of karyopyknosis when one screens the specimens with the 10x magnification. The phenomenon is not present when one uses the oil immersion objective, but may also be observed on unfixed and unstained smears.

SUMMARY

Summarizing this presentation of the advantages and disadvantages of phasemicroscopy for routine diagnostic purposes, one may say that the examination of unfixed and unstained cytological slides is not a substitute for the Papanicolaou method; it may supplement it, however, either as a prescreening technique or as an additional screening technique. The phasemicroscopic examination of unfixed material as an additional technique performed in the outpatient department is of real advantage. It also seems to be the method of choice for teaching institutions. The greater cellular detail of the unfixed and unstained cells has no bearing on the routine cytological

diagnostic procedure. The phasemicroscope may be used successfully for photomicrography on stained and fixed cells if the proper type of microscope is selected. The phasemicroscope may also be used with success for an objective standardization of the Karyopyknotic Index, an important factor in hormonal evaluation.

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DISCUSSION

GUNTER F. BAHR, Stockholm, Sweden:

Comparing the routine inspection of unfixed smears under the phasemicroscope with the screening of fixed and stained smears, a point made by Wied seems to deserve particular attention: hyperchromasia or any tinctorial phenomenon is not related to the phase image. For the purpose of this discussion we consider the latter roughly dependent on variations in the concentration of organic substances (and the related refractive index).

Hyperchromasia, too, is primarily reflecting concentration. However, since the binding of hematoxylin is mainly a chemical process involving acid groups, it is the presence and number of binding sites that decides the uptake of the dye. In many instances, though, the amount of bound dye will roughly parallel the amount of organic substances present, but this does not necessarily have to be so.

JACQUES FERIN, Louvain, Belgium:

I agree completely with Wied. For many years, to our greatest satisfaction, we have used phasemicroscopy on unfixed and unstained material as an additional routine office technique for hormonal and microbiological classification.

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CAMILLE J. P. LICHTFUS, Strasbourg, France:

I wish to emphasize the use of phase contrast microscopy in three cases:

1) In threatened abortions: At the time of admission, the unfixed vaginal smear is examined under the phasemicroscope with regard to increase in the Karyopyknotic Index, etc., giving us information as to the seriousness of the possible hormonal disturbance. Following conservative treatment, each patient is followed biweekly by a vaginal smear, which is examined fresh for determination of the level of estrogens. The therapy is adjusted according to the cytological picture.

2) At term: It permits us to discover infection and cytolysis easily. These two possibly prevent cytological determinations of the hormonal condition and may make it necessary to perform a local preliminary anti-infestation treatment.

3) With my teacher Pundel, we perform the determinations of the Karyopyknotic Index with the aid of phase contrast microscopy on fresh specimens or specimens stained after fixation. The method is very simple and very precise.

PETER STOLL, Heidelberg, Germany:

One should consider the values of phasemicroscopy from both a cytological and a clinical standpoint.

1. The scientific possibilities are not yet fully appreciated. We see in the cytoplasm, as well as in the karyoplasm and in the cellular and nuclear membranes, structures which change

during the differentiation stages of the cell and are not yet completely known and evaluated. These structures vanish after fixation and staining procedures.

Here are some examples:

a) The behavior of living cells in tissue culture, intracellular changes, mitoses.

b) The behavior of living cells in different media, diffusion processes, localization of the sites of deposition and fixation of vital stains.

c) Observation of structural changes under different functional conditions, i.e., cytoplasmic granules during the menstrual cycle in squamous epithelia.

d) Disorganization of the plasmatic structure in carcinoma cells.

e) Changes in the cellular membranes, i.e., intercellular bridges.

f) Motion studies on ciliated epithelial, for instance from the cervical canal or from the tubal tract. Examination of ciliated cells from ascitic fluid and from ovarian cystomas.

g) Application of the method as pre-screening for electron microscopic observations.

2. For practical purposes in gynecology, answers may be obtained as to the following:

a) Functional situation in the vaginal smear.

b) Vaginal flora, especially trichomonas with their flagellate movements, and also cystic forms. Besides, the vaginal bacterial flora may well be differentiated. "Döderlein, Streptococci, Staphylococci, Bacteria Coli, Spirillae, Spirochetae and Fungus Diseases."

c) Leukocytes and erythrocytes.

d) The presence and motility of sperm in the cervical secretion in infertility consultations.

e) The evaluation of abdominal taps, fluid from ovarian tumors, puncture specimens from the cul de sac for erythrocytes (intra-abdominal bleeding).

Where cancer detection is concerned, phase contrast shall not and cannot replace the Papanicolaou technique. Only the stained preparation can be screened in the laboratory with plenty of time and suspicious cells are recognized much easier by their hyperchromasia.

The advantage of the phase contrast method in gynecology is the possibility offered for the immediate diagnosis at the time of examination: according to the functional situation and/or the vaginal flora, decisions as to the treatment can be made at once. Especially in patients with vaginal discharge, the repeated control of the treatment is done quickly and safely without any other aids.

The preparation of the smear is simple: two drops of normal saline are put on a slide, one to receive the vaginal material, the other for the cervical specimen, which are obtained with the aid of a platinum loop. The slide is covered with a cover glass and ready for screening. No higher power than 16x6 or 40x6 is recommended. There shall be no search for carcinoma cells.

Our practical procedure is as follows:

A. Patients with Vaginal Discharge

Out-patient Department
1. Consultation

Phase---------- Speculum examination ---------- Papanicolaou
(Office) (Labor)
Function Function
Flora
Leukocytes Carcinoma cells
Erythrocytes
Sperm

Phase----------2. Consultation
Phase----------3. and Flora Treatment
Phase----------4. with Controls
Phase----------... ----------after 6 months repetition
Papanicolaou
(Labor)

The phase contrast preparation is prepared by an aid (student); the reading itself does not take longer than 30 seconds since it is for orientation only. The patient is still on the table and the "guided" treatment can be started immediately.

In this, I fully agree with Wied's table III and refuse the phase contrast microscopy for pre-screening in cancer diagnosis.

B. Sterility

Vaginal Secretion	Cervical Secretion
Phase Microscope	Phase Microscope
Function	Leukocytes
Flora	Sperm
Sperm	Crystallization

C. Differential Diagnosis

Vaginal Secretion	Cervical Secretion
Phase	Phase
Function	Leukocytes (with ascension)
Flora	Erythrocytes (with ascension and Abortus febrile)
Leukocytes	
Erythrocytes	

D. Acute Abdomen

Douglas' spot

Phase

Erythrocytes	(Bleeding: Extra-uterine pregnancy)
Leukocytes	(Peritonitis)
Tumor Cells	(Tumor Ascites)

Again I emphasize that I personally prefer the Papanicolaou technique for purposes of cancer detection since (1) phase contrast is a time-consuming measure and (2) good color contrast is required. Therefore, I believe that the gynecologist should take a regular vaginal smear once every 6 months, but use the phasemicroscope any time, since he will get valuable information concerning the treatment of his patient.

HANS KLAUS ZINSER, Cologne, Germany:

Phase contrast microscopy can be used successfully in examining fixed and stained material, and many structural items are surprisingly well pronounced. However, the method of studying living objects is particularly useful. Since only very thin layers allow optimal working conditions, excretes and exudates are particularly the best prerequisites for phase contrast observations. Therefore, the limits for extensive clinical use are quite wide. The numerous possibilities of this "vital microscopy" have been referred to in an extensive survey published in the "Klinische Fortschritte in der Gynäkologie," pg. 181-208, Urban and Schwarzenberg, Wien, publishers.

Wied's study concerning the pros and cons of phase contrast microscopy has my full agreement. For routine work, the advantage is that the cytological evaluation of a given specimen can be done immediately, without time-consuming lab techniques. But this "rapid test" must be supported by sufficient experience in evaluation of vital cytological pictures. Even cytologists familiar with the features of fixed and stained preparations need more extensive training to safely judge unusual findings. Thus I consider it doubtful to recommend this method for use in general practice, since, as stated before, more specialized knowledge is necessary in order not to endanger the accuracy of the diagnosis. As a rule this method will likely only be used under clinical conditions which provide the above mentioned prerequisites.

As a substitute for the classical Papanicolaou stain, phase contrast microscopy is thus far only of value in separating the unsuspicious specimens from a large number of smears. Then one can have the suspicious and positive ones fixed and stained after Papanicolaou. This pre-selective method takes away much of the routine work from overloaded cytological labs with inadequate personnel. Making a fresh preparation is very simple: a drop of material is placed on the slide, covered and squeezed between two glass plates to provide equal distribution of the material for

observations. There is no need for the use of oil immersion. The application of efficient "dry systems" is recommended to allow more rapid screening of the specimen. The handling of modern phase contrast microscopy (Zeiss, Leitz) is entirely uncomplicated. Effortlessly a bright field as well as a phase contrast observation can be accomplished. Zeiss has a new device called "Mikromat," which permits rapid taking of photographs due to its built-in photographic equipment.

Obviously, the desirability of fresh preparations is limited; therefore, we store the slide in a moist chamber until screening. By framing the slide with paraffin, we are able to obtain slides which can even be examined after one day's duration. Thus, questionable findings can later be presented to better trained men for the final diagnostic decision.

In spite of advantages described above, the Papanicolaou technique must be preferred for routine diagnostic work since there is a need for documentary material, which can be obtained only with this technique. This is true for hormonal evaluation as well as for cancer diagnosis.

However, I cannot agree with the statement that phase contrast microscopy usually offers less reliability than screening with classically fixed and stained preparations. With proper proceedings, the phase contrast image offers a series of morphological details, which are not recognizable in the congealed "snap-shot" of the fixed and stained smear. This applies particularly to the nucleus and the cytoplasmic constituents.

It is obvious, moreover, that these biological and pathobiological cellular processes which are only comprehended in the vital cytological pictures are recorded. Furthermore, the nonapparent hyperchromasia is considered a disadvantage. It may be said that this staining peculiarity corresponds to the typical bright appearance of the nucleus in the phase contrast image.

However, it must not be denied that phase contrast microscopy predominantly is an aid for the basic science. Many of the phase optical findings on the cell constituents have mere theoretical value and cannot be used for the practical diagnosis.

CLOSING REMARKS

GEORGE L. WIED, Chicago, Illinois, U.S.A.:

May I thank Drs. Bahr, Ferin, Lichtfus, Stoll and Zinser for their discussions.

I am in agreement with Bahr that the light appearance of nuclei under the phasemicroscope only signifies that the nuclei are dense, but not necessarily hyperchromatic as Zinser has suggested. Density and hyperchromasia are not necessarily identical.

Several of the points raised by Drs. Stoll and Zinser go beyond the scope of the present subject, which is only concerned with the use of phasemicroscopy in routine cytological evaluations. The seven examples given by Stoll in the first paragraph of his discussion belong in this non-routine or research application of phasemicroscopy. Similarly, Zinser mentions that phasemicroscopic examination permits the identification of various cellular and nuclear details which cannot be discerned with the bright field microscope on the fixed and stained specimen. I do agree fully with that statement; however, within the scope of the present subject, it does not mean too much that the phasemicroscope permits identification of cellular and nuclear details which are of no practical importance for cytological diagnosis, with the present stage of knowledge.

The statement by Zinser that modern phasemicroscopes (by Zeiss and Leitz) are completely uncomplicated to handle deserves a little qualification. I would define as "completely uncomplicated" a phasemicroscope which can be switched without readjustment from the 10x to 40x to 100x magnifications and vice versa after it has been adjusted once for phase, i.e., that all three phase rings would be permanently, or at least for many hours, aligned correctly. A further qualification would be that one could switch the instrument from phase to bright field and to phase again without readjustment of the phase rings. This is certainly not true for the phase system of Leitz, in which the adjustment is made by lowering and raising the condenser; for the most expensive microscope of Zeiss-VEB-Jena (Lumipan), where the adjustment is made by turning the pancratic condenser or, finally, for the Zeiss-Winkel microscope. However, it was and is possible with the rather old phase condenser of Zeiss-VEB-Jena which was being mounted on their LG- and LU-models (as far back as 1949) and with the expensive microscope of Zeiss-Opton (as far back as 1951). The cytologist who wishes to use his phasemicroscope for routine work should, therefore, consider very carefully which model offers him the least time-consuming work. In this respect, e.g., the less expensive LG- and LU-models of Zeiss-VEB-Jena are superior to the more expensive Lumipan-model of the same factory, although the Lumipan-model might offer other advantages which are not under consideration here.

The most automatic photomicroscope can never record as many representative photographs for the permanent record as is possible with having a fixed and stained specimen on file. With the total expenditure for 10 photographs, which is a minimum for each individual fresh smear,

one could easily pay for fixing and staining 100 specimens in an established cytology laboratory. In addition to the expense, one must consider the fact that the fresh cells usually move in their suspending medium and are often blurred on the photograph and that the filing of photographs is much more "non-routine" than the simple filing of a fixed and stained specimen.

Concerning the comparative diagnostic accuracy, it is stated by Zinser that he does not agree that phasemicroscopy on fresh specimens has a lower diagnostic accuracy than examination of the fixed and stained specimen. In my opinion, this statement also needs further qualification. If Zinser had said that phasemicroscopy has not a lower accuracy in selecting the entirely normal smears from those which need further examination, one could have agreed. The (fresh) entirely normal cells can be properly identified as such, but as soon as the words "diagnostic accuracy" are interjected into the discussion, one must assume that the discussant means, e.g., that using either technique one is able with the same degree of accuracy to distinguish a benign dysplasia from an incipient carcinoma. This differentiation to a high degree of accuracy is certainly possible by means of the fixed and stained smear. Here phasemicroscopy has a definitely lower accuracy than bright field microscopy on the fixed and stained smear. The inability to differentiate hyperchromatic nuclei alone is a definite drawback of the phase technique which cannot be compensated for—as Bahr has pointed out—by the fact that dense nuclei appear light under the phasemicroscope.

In summary, I remain convinced that phasemicroscopy on fresh specimens is less economical, more time-consuming, more fatiguing, more difficult to learn, less practical and less accurate than the Papanicolaou technique for routine cytological examinations and that it will not replace the Papanicolaou technique. Phasemicroscopy may have its place as a "pre-selecting technique" after proper training of the interpreter, and it may be useful as an additional "immediate interpretation" of hormonal condition and/or for microbiological classification, as used by Ferin, Lichtfus, Stoll and by ourselves. For the latter purpose phasemicroscopy on fresh specimens seems to be an excellent substitute for any vital staining procedure in routine diagnostic work. One can agree with the discussants who state that phasemicroscopy on fresh specimens reveals nuclear and cytoplasmic details which are lost due to fixation and staining. However, one fails to find any advantage in the visualization of these additional details for routine cytological diagnostic work, until somebody shows convincingly that these additional details retain any diagnostic significance.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

ADVANTAGES AND DISADVANTAGES OF THE ANOPTRALMICROSCOPIC PROCEDURE IN EXFOLIATIVE CYTOLOGY

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In 1953 the Finnish scientist, A. Wilska, introduced a new method of obtaining contrast in the microscopic images (1, 2, 3). The optical company, C. Reichert A. G., Vienna 17, Hernalser Hauptstr. 219, Austria, in collaboration with Wilska, has designed a special device called "anoptral." Working with this equipment, which consists of a special condensor and anoptral objectives, is very simple. Adjustment is obtained with the aid of an auxillary microscope, as in phase contrast microscopy. The microscopic image appears in a honey-colored light.

The accompanying pictures were taken with a plate camera attached to Reichert's Zetopan microscope. Oil immersion and the "Polyphos" condensor were used. The condensor permits a continuous transition from a bright field, over the anoptral effect, to the dark field. The particular adjustment of the condensor is reproducible. Reichert's Cirkonium lamp, which gives a very bright light and allows short exposure time, was used as a source of illumination.

Anoptralmicroscopy exhibits a similar, however, not identical effect as negative phase contrast microscopy. The resulting contrast and resolution of anoptralmicroscopy is, especially in high magnification, better than with the phase microscope.

First the preparation of the material: the material demonstrated in the figures has been obtained with an ordinary wooden spatula. The specimen was immediately smeared on a glass slide and embedded in tyrode solution. The microscopic examination should take place as soon as possible since the cells undergo autolytic changes more or less rapidly. For smears from carcinoma the following alternative technique for preparation of the specimens is recommended: the material is again obtained with an ordinary wooden spatula, but the spatula is washed in a tube filled with tyrode solution. The tube is put into a centrifuge and a droplet of the sediment is examined under the anoptralmicroscope. The cells are not damaged with this procedure.

Phasemicroscopy and anoptralmicroscopy have great scientific value since they permit observation of the vital structure of the cells. For the routine examination of smears such as for cytological screening for carcinoma I believe, however, that both techniques are not suitable: the preparation of the specimens is by no means less complicated than the staining of the smears according to the Papanicolaou technique in an established cytology laboratory. The evaluations of the specimens under the anoptral and phase microscopes are considerably more difficult because of the abundance of visualized cellular details as compared with the stained smear. There will always be some cellular details which cannot be interpreted even by the experienced reader. False negative and false positive interpretations with the unfixed and unstained material cannot be avoided. For example, those nuclei which appear hyperchromatic in the stained smear often do not exhibit a visible contrast. On the other hand, in the cytoplasm of the unfixed and unstained material one sees small droplet-like inclusions and little clods which are never visible -- not even slightly indicated -- in this amount in the stained cells.

For the cytologist the observation of the cells with both anoptral or phase microscopes is nevertheless very interesting, because he can deepen his knowledge about cellular morphology. The one who is not familiar with the histology of the tissue from which the smears are prepared and who is not familiar with the vital structure of the cells which he sees in the stained smear, actually speaks with the same authority as does the blind man when talking about color. Setting aside the

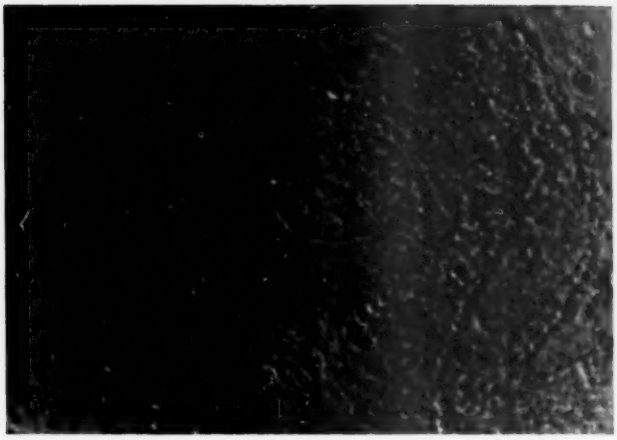

Fig. 1. Vaginal smear of healty woman (multipara) on the seventh day of the menstrual cycle. Bright field microscope, with closed condenser diaphragm.

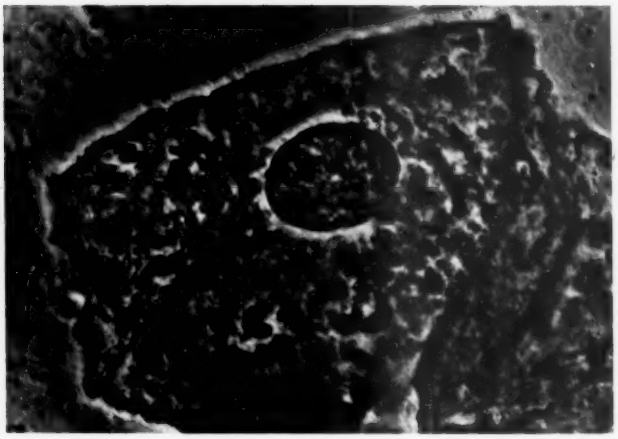

Fig. 2. Same cell as Fig. 1. under the phase microscope.

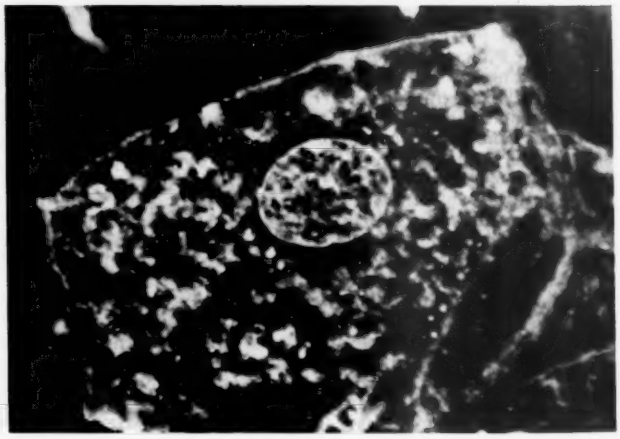

Fig. 3. Same cell as Fig. 1. under the anoptral microscope.

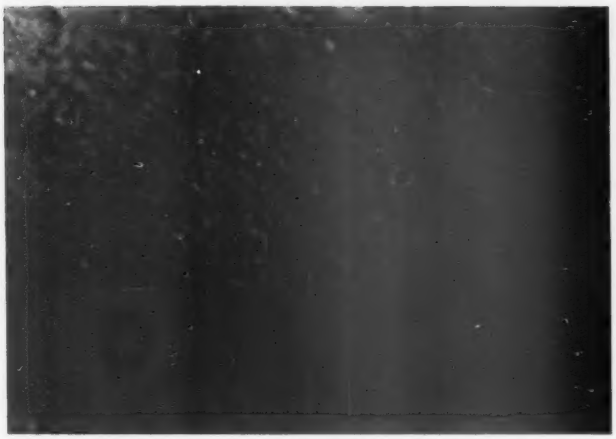

Fig. 4. Cervical smear from the squamo-columnar junction of the same woman as depicted in Fig. 1: endocervical cell under the bright field microscope.

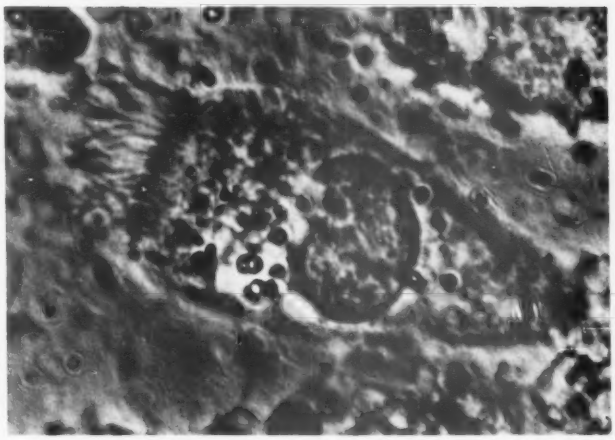

Fig. 5. Same cell as Fig. 4. under the phase microscope.

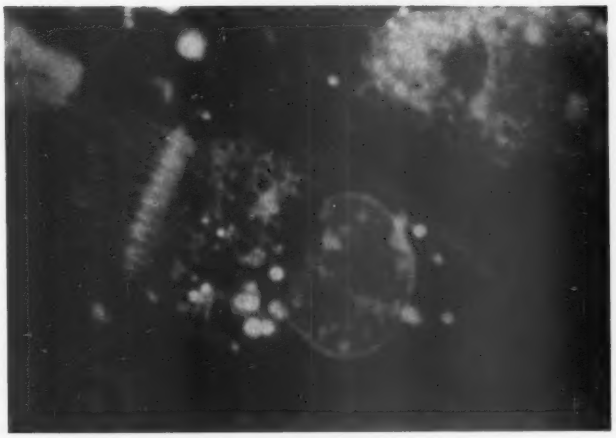

Fig. 6. Same cell as Fig. 4. under the anoptral microscope.

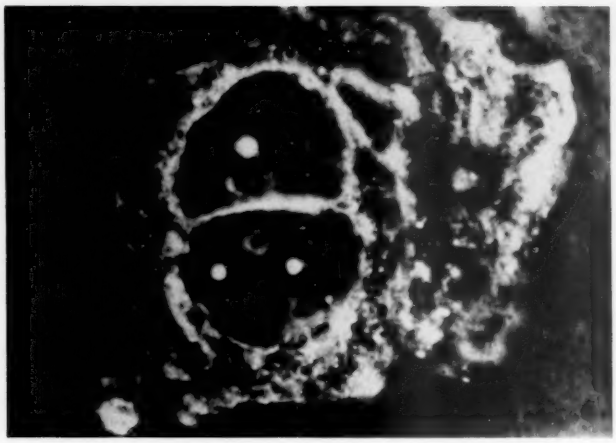

Fig. 7. Cervical smear of a 43 year-old patient with carcinoma in situ. Anoptral microscope.

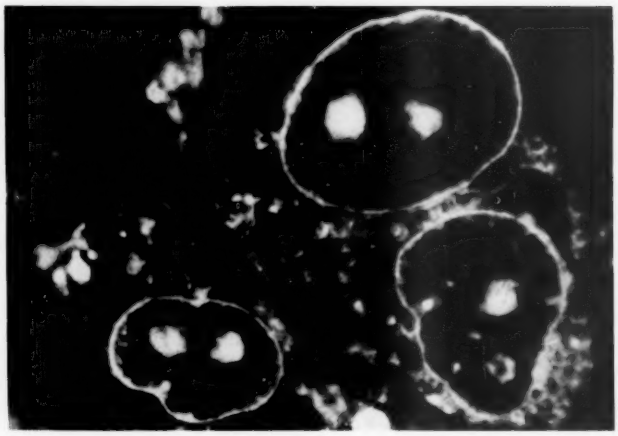

Fig. 8. Cervical smear of a 36 year-old patient with poorly differentiated solid cervical carcinoma. Anoptral microscope.

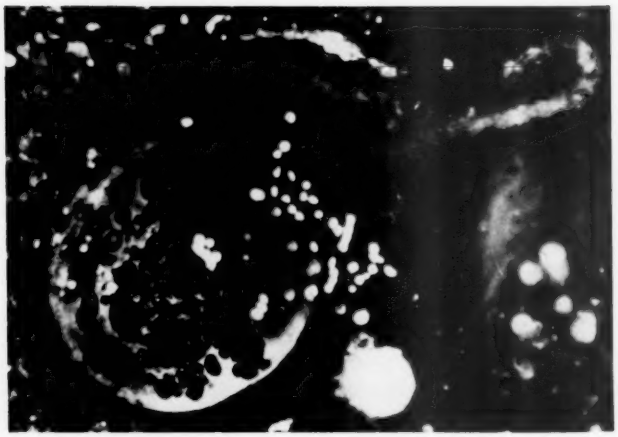

Fig. 9. Cervical smear of a 48 year-old patient with exophytic cervical squamous carcinoma. Anoptral microscope.

other scientific applications seems to me to be the significance of anoptral- and phase microscopy for the practicing cytologist.

In regard to the figures, I would like to mention the following:

Figs. 1-3 demonstrate normal superficial squamous epithelial cells of the vagina. With the bright field microscope one can only distinguish the cellular and nuclear contours. With the phase microscope cytoplasmic and nuclear details become visible. The margins of the arched cell become necessarily blurred due to the small focal depth with high magnification. Close to the nuclear membrane is a chromatin body, apparently the sex chromatin. The demonstration of the cell with the anoptral microscope seems to be superior to the phase microscopic photograph.

Figs. 4-6 show an endocervical cell with kinocilia. The bright field microscope again permits only the demonstration of the contours of the cell. Only when applying the phase or anoptral microscope does one see details of the cell which is filled with secretion. Numerous droplets of secretion lie in, around or on top of the cell. The tyrode solution which surrounds the cell is isotonic but does not mix with the viscous cervical mucus-resulting in formation of streaks which are especially disturbing under the phase microscope. Under the anoptral microscope the droplets of secretions appear very bright. The cilia sit on basal knots and move vivaciously. On the living cell one can discern the "roots" of the cilia which are directed to a center exhibiting systolic contractions.

Figs. 7-9 show cancer cells under the anoptral microscope. Fig. 7 is a binucleated carcinoma cell with well preserved cytoplasm, and exhibiting a large nucleolus. Cytoplasmic and nuclear details can be well identified. The anoptral contrast gives a plastic impression of the cell. Fig. 8 shows anisonucleosis and nuclear polymorphism in a smear from a poorly differentiated solid carcinoma of the uterine cervix. Figure 9 shows a tadpole cell with mitosis from a differentiated epidermoid carcinoma.

In summary, I would like to say that anoptral microscopy has no practical value for the cytological diagnosis, as is the case with phase microscopy. The practicing cytologist will, however, have the opportunity to observe the vital structure of the cells, which otherwise he sees only after fixation and staining. For the field of cellular research both anoptral and phase microscopy are of great importance. One may mention here only tissue culture work which necessitates the usage of a microscope permitting vital cell observation. Anoptral microscopy seems to be superior to phase microscopy when using high magnification.

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DISCUSSION

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

I would like to congratulate Schüller on his fine examinations and also on his demonstration of the possibilities and limitations of the technique. I would also like to take this opportunity to ask him whether or not he has made observations with anoptralmicroscopy as to the duration of mitosis, and if he could observe how mitoses occurred in normal and pathological nuclei. I would also like to ask whether or not he could observe the continuation of mitoses which were already in progress. It is often difficult to distinguish in the fixed and stained specimen if a cell exhibiting mitosis belongs to the histiocytic or epithelial group. In smears from carcinoma one finds mitoses only very rarely, whereas they may be detected rather often in the corresponding histological sections. In sections the presence of mitoses even represents a diagnostic criterion. Can anoptral microscopy contribute something to the explanation of this observation?

PETER STOLL, Heidelberg, Germany:

Schüller has discussed very thoroughly the value of anoptral microscopic and phase microscopic examination of unfixed and unstained cytological specimens. It seems to me that the scientific value of the techniques may lay especially in the demonstration of cytoplasmic detail, the importance of which is not very much investigated as yet for hormonal and cancer diagnosis. The vital cytological examination and investigation form a bridge to electron microscopy. I am in agreement with Schüller that the practical value of these techniques for carcinoma detection is not very great. The immediate evaluation of vaginal flora and hormonal patterns, however, seems to me a definite advantage. In this application the technical approach of both phasemicroscopy and anoptralmicroscopy is identical. Probably one uses the technique with which he first becomes acquainted.

CLOSING REMARKS

EDMUND SCHÜLLER, Wien, Austria:

It does not seem justified to advise a practicing gynecologist to purchase an anoptral (or phase) attachment for his microscope. It is certainly true that one can diagnose Trichomonas vaginalis excellently with these special microscopes, but the definite identification of the bacterial flora seems problematical to me, since these techniques do not offer the possibility to differentiate gram positive from gram negative bacteria, which is often the decisive factor in bacteriological diagnosis. Admittedly the experienced interpreter can correctly evaluate the smears under the phase or anoptral microscopes, but with greater effort and more time than with stained disk.

As far as the scientific value of the methods is concerned it may be said that they are in a certain degree superior to electron microscopy because only with these methods can one observe the vital structure of the cells. Photography cannot demonstrate what is observed under the phase or anoptral microscope, namely the movement of the kinocilia. There are considerable difficulties in demonstration of the movement by means of moving pictures, especially because these examinations require high magnifications which pose marked technical difficulties.

We could not observe the individual phases of mitosis with the anoptral procedure. We would have needed a heated stage on the microscope for this study. However, further technical difficulties may arise - for instance, by evaporation of the thyrode solution. Furthermore, it is questionable whether or not the mitosis will be disturbed by extensive exposure to light.

Whether or not a mitotic cell belongs to the histiocytic or epithelial class, can be recognized even less easily in anoptral contrast than in the stained preparation. One rarely finds mitoses in fresh preparations, even though they are taken from a carcinoma which exhibits many mitoses. This is due to the fact that the mitotic cell is extremely fragile and almost always will be destroyed by the smear procedure. I will soon publish a series of reproductions of mitoses in fresh preparations. To get only a few useful pictures, it took me almost two years and I had to examine many hundreds of smears.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

ADVANTAGES AND DISADVANTAGES OF THE INTERFERENCE MICROSCOPIC PROCEDURE IN EXFOLIATIVE CYTOLOGY

WALTER SANDRITTER
Frankfurt a.M., Germany

It is possible to determine by means of the interference microscope the dry weight of individual cells or cellular constituents. The principle of interference microscopy is based on the fact that a beam of light which goes through a dense medium (object) undergoes a retardation of its phase as compared to a reference beam. The degree of the retardation depends on the concentration of the substance (refraction) and the thickness of the object. If one examines the two beams with optical systems the interference appears in the form of an increase or decrease of the intensity of the light (1, 2, 3, 6).

The above principle has been employed in the construction of Baker's Interference Microscope (analyzator method) where the measurements are made with an analyzator by means of bright and dark adjustment of the structures of the object and the comparative field.

The general formula for the determination of the dry weight is:

$M = \frac{\phi m \cdot F}{\chi}$: ϕ m is the phase difference, F is the area and χ is the "specific increment," 0.18. (For more detailed information see reference number 5.) Other interferomicroscopic principles, such as the fringe method (Baker and Dyson microscopes), cannot be used for routine cytological work because of the complexity of the technique.

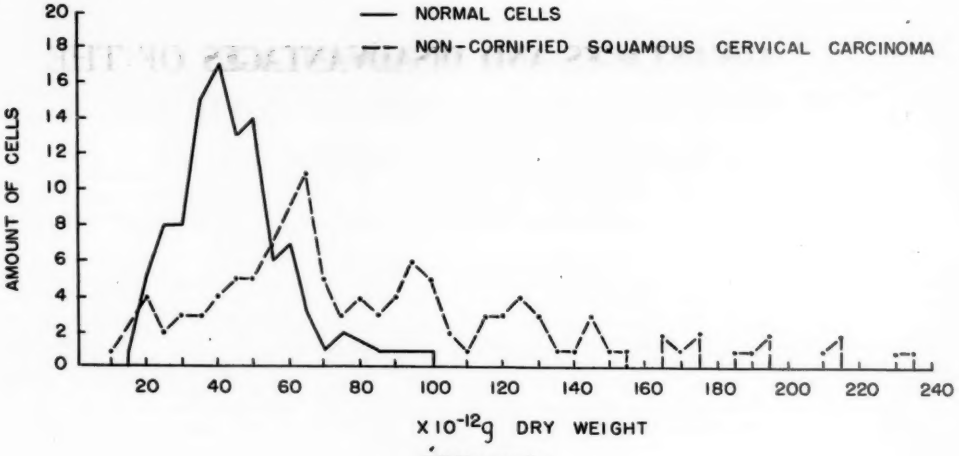
The advantage of the interference microscope over the phase microscope is that, besides giving excellent contrast on unfixed and unstained specimens under monochromatic light (permitting analysis of the cellular structure), it is possible to determine the dry weight. The degree of resolution corresponds favorably with that of a bright field microscope.

However, the interference microscope has the same disadvantages as the phase microscope, namely, that the specimen per se is not permanent. In addition, with the Baker interference microscope, the condensor must be changed each time one changes the magnification. Thus, it is not easy to interchange the objective during examination to study this or that cellular detail more carefully, and one must confine one's observation to either 40x or 100x. A further disadvantage is that the cellular features may appear disturbed, especially by larger cellular constituents, due to the Shearing-effect.

With these things in mind, we ask ourselves whether or not interference microscopy would prove a useful addition to routine cytological diagnostic work. In order to answer this question, we performed dry weight determinations on normal and cancer cells. Table I shows the distribution of the dry weight values on 102 normal cellular nuclei exfoliated from the vaginal epithelium compared with 117 cells exfoliated from an immature cell-type squamous carcinoma. One notes that the nuclei of the carcinoma cells generally exhibit an increased dry weight (mean value 90.78 x 10 $^{-12}$g, as compared with normal cellular nuclei of 42.97 x 10^{-12}g). If one would demarcate normal from malignant cells at 55 x 10^{-12}g, then in this case 70% of the carcinoma cellular nuclei show an increased dry weight (of over 55 x 10^{-12}g), whereas only 20% of the normal nuclei lie above this arbitrary border. The evaluation expressed in percentage of cellular nuclei above 55 x 10^{-12}g of 10 cases with carcinoma (squamous carcinoma, small cell carcinoma, adenocarcinoma) exhibited the following results: case #1: 50%, case #2: 45%, case #3: 100%, case #4: 25.7%, case #5: 27.4%,

case #6: 95.5%, case #7: 70%, case #8: 96.5%, case #9: 71.4%, case #10: 91%, and case #11: 100% (4). This preliminary experiment, which has to be verified further by more extensive studies, shows clearly that the nuclei of carcinoma cells have a greater dry weight than nuclei of normal cells.

For routine work this technique is certainly too time-consuming (especially the determination of the cell area). Increased efficiency could be achieved by using calibrated ocular rings or automatic computator. Also, the use of color interference microscopy with polychromatic light, where structures with higher dry weight concentrations are selectively colored, still offers many possibilities.



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DISCUSSION

ALVAN G. FORAKER, Jacksonville, Florida, U.S.A.:

It is rather unlikely that interference microscopy will ever play an important role in diagnostic cytology, as now practiced. In addition to factors mentioned above, other disadvantages are the comparatively great expense of the instrument, its inherent complexity, and the time involved in mastering and employing the technic. To be of maximum value in the total study of the patient, clinical as well as cytological, preparations should be part of the permanent record. They should be readily available for reexamination months or years later. They should be reviewed in context with subsequent cytological or histological studies, and with clinical findings. Special research technics such as interference or phase mircoscopy do not compare with fixed and stained smears in utility, considering economy of time, effort and materials.

However, preparations for examination by interference microscopy need not be impermanent. It is quite possible for fixed smears to be examined, then stored for reference. A routinely prepared, alcohol-ether fixed, but unstained smear can be mounted in a medium of suitable refractive index (e.g., 1.45 or 1.60) and examined by interference microscopy as desired. The choice of mounting medium depends on the type of study. However, mounting medium with a refractive index too close to that of tissue (about 1.54) should be avoided.

Nuclei of malignant squamous cells in preliminary studies reported by Mellors (3), do seem to have greater dry weight than those of normal cells, as indicated by Sandritter. Yet one wonders if this is not more a function of variation in size, rather than dry mass per cubic micron of nucleus. Nuclear dry weight according to the general formula quoted by Sandritter is the product of phase difference and area divided by the "specific increment." It would be interesting to see the mean values for the nuclear areas, and for the dry mass per square micron of area, used in computing Table I. In Sandritter's data the mean value for dry weight of nuclei of carcinoma cells

was 2.11 times that for normal cells. In one of our own studies on tissue sections (1) nuclei from the "superficial layer" of invasive carcinomatous epithelium had mean nuclear area 2.47 times that of nuclei from the superficial layer of normal exocervical squamous mucosa from the same cases. Currently we are evaluating data from an interference microscopic comparison of nuclear mass and allied phenomena in sections showing normal exocervical mucosa and abnormal cervical epithelium (metaplasia, dysplasia, intraepithelial and invasive carcinoma). A preliminary report has been made (2). Mean nuclear weight, from the "midzone" of invasive squamous cell carcinoma was 1.79 times that for nuclei from the comparable zone of normal exocervical mucosa from the same cases. However, the mean area of the same carcinomatous nuclei was 1.85 times that of the comparable normal nuclei. The dry mass per square micron of these two types of nuclei was virtually identical. This finding casts additional doubt on potentialities of interference microscopy for routine diagnostic cytology.

The interference contrast microscope certainly is an interesting and potentially valuable research tool. It probably will play little part in routine cytology in the foreseeable future.

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ROBERT C. MELLORS, New York, N.Y., U.S.A.:

Sandritter has concisely presented his preliminary experience in the study of exfoliated cells in the vaginal smear with quantitative interference microscopy. The preliminary experiments show that the nuclei of carcinoma cells (squamous carcinoma, small-cell carcinoma, adenocarcinoma) have a greater dry weight than nuclei of normal cells. I have obtained somewhat similar results in work carried out several years ago, although the absolute magnitudes of nuclear mass were appreciably higher. In this early work I asked myself the question, which I now pass on to Dr. Sandritter, namely, how does one estimate the nuclear mass in a cell when the nucleus is overlain and underlain by cytoplasm of unknown mass? The report of Fitzgerald on applications of x-ray microscopy to the measurement of nuclear and cytoplasmic mass in sections of human uteri (Ann. N. Y. Acad. Sci., Vol. 63, Art. 6, 1141-1176, 1956), should also be cited. In that report, examples of cervical carcinoma in situ are described in which the nuclei of carcinoma cells had less mass concentration than did adjacent normal cervical epithelium.

CLOSING REMARKS

WALTER SANDRITTER, Frankfurt, a.M., Germany:

I am in complete agreement with Mellors' observation that the cytoplasm above and below the cell nucleus introduces an error which cannot be eliminated. Considering, however, the mechanical compression which the cytoplasm is subjected to, it is my opinion that this error may be disregarded for all practical purposes.

We have obtained results identical to Fitzgerald and Foraker regarding the dry-weight concentration. The percentual dry-weight content (resp. the dry weight concentration) of the carcinoma cell nucleus and the cytoplasm is less than that of the normal cell.

Nucleus	
Carcinoma cell	6.7%
Normal cell	10.8%
Cytoplasm	
Carcinoma cell	2.0%
Normal cell	4.89%.

The increased dry weight of the cancer cell is determined by the size of the cell. The cancer cell contains more water than the normal cell.

I am in agreement with Foraker that the use of the interference microscope for routine purposes is impractical because of the difficulties encountered. These difficulties are, in principle, the same as those encountered when using the phase contrast microscope.

Regarding slide fixation, I would like to point out that the specific increment of 0.18 is definitely altered after alcohol fixation. This value is valid only for undenaturated proteins. The specific increment is unknown for alcohol-fixed proteins.

ADVANTAGES AND DISADVANTAGES OF THE GALLOCYANIN CHROME ALUM PROCEDURE IN EXFOLIATIVE CYTOLOGY

WALTER SANDRITTER
Frankfurt a.M., Germany

The dye gallocyanin chrome alum combines with both nucleic acids (RNA and DNA) and results in a blue nucleic-acid-and-dye-complex (progressive staining procedure) (1, 2, 5).

Technique: The smears are fixed in 96% alcohol for 10 minutes while still wet. Preparation of the dye: 5 g of chrome alum puriss is dissolved in 100 cc. distilled water; 0.15 g of gallocyanin (Fluka) is added and the mixture is heated slowly. The mixture is brought to a pH of 1.64 with either HCl or NaOH. The solution may be used for about three weeks. The actual staining procedure: 48 hours staining in this solution, and differentiating in ascending alcohols. The times are not of great importance, because the staining procedure is progressive. After staining, the slides are covered and ready for examination.

The advantages of the Gallocyanin-Chrome-Alum-Procedure are in its progressive character and the resulting reproducibility. The nucleic acids of the nucleus (RNA and DNA) and of the cytoplasm (RNA) are demonstrated quantitatively. The degree of "basophilia" of the cellular elements is, therefore, in the critical factor in the qualitative evaluation of these cytological smears.

The disadvantages are:

(1) The rather lengthy staining procedure. This can be shortened, however, by staining the smears at a higher temperature (60° C.). Investigations on this are still in progress.

(2) Both nucleic acids stain blue. RNA and DNA can be differentiated only after comparison with an additional control specimen treated with ribonuclease.

(3) The procedure is not entirely specific since mucus and calcium are also stained.

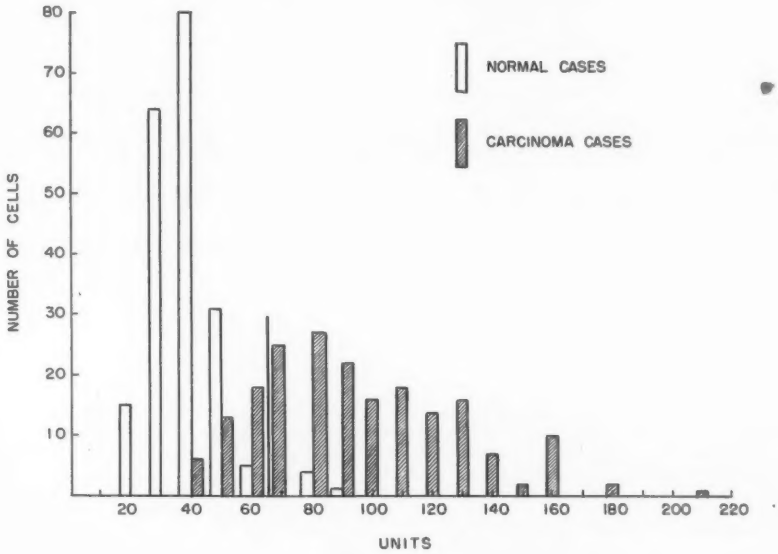
The quantitative character of the staining procedure permits the evaluation of the nucleic acid contents of the nuclei by means of photometry. In previous experiments (6) it has been shown that malignant tumor cell nuclei exhibit a markedly increased content of both nucleic acids compared to the content in the surrounding tissue (in 48 out of 50 cases). Cytophotometric examinations of exfoliated vaginal epithelial cells showed that normal smears (Table I) exhibit only a maximum of 10% of the nuclei with a nucleic acid content which lies above a distinct limit (Limit: 65 AE, u^2 x intermediate extinction, measured with filter 497 mμ). In certain cases of carcinoma one finds, however, always more than 10% of the nuclei with an increased nucleic acid content (over 65 AE). In four observed cases an average of 82% of the cells contained nuclei with a nucleic acid level of above 65 AE.

In order to investigate the efficiency of cytophotometric smear examination as a routine procedure, a larger series of cases should be evaluated (see also 4 and 7).

Test examination diagnosing smears from patients where the actual histological diagnosis was unknown to the author were successful. These tests were performed in cooperation with the Department of Obstetrics and Gynecology of the University of Frankfurt (Dr. Cramer). Dr. Cramer evaluated the smears fixed and stained according to the Papanicolaou technique ("subjectively") while this writer evaluated them using the above gallocyanin chrome alum technique ("objectively").

* with support of the Deutsche Forschungsgemeinschaft

In the eight cases which were reported by routine clinical technique as negative, a maximum of 6% of the nuclei exhibited an increased content of nucleic acids. Five cases of carcinoma showed an increased content of nucleic acids (case #1: 34%, case #2: 34%, case #3: 36%, case #4: 16%, and case #5: 28%) in over 10% of the cells. A cytological smear which was subjectively classified as Class III (suspicious) showed in 18% of the cells an increase in the amount of nucleic acids present.



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DISCUSSION

GUNTER F. BAHR, Stockholm, Sweden:

For some years Sandritter has applied the gallocyanin stain to cytochemical problems. From his work and the previous work of Einarson it is clear that cytochemistry has a valuable tool in this stain. I think that it should be applied to cytochemical problems in a larger extent than is now the case. Used in combination with nucleases it is certainly comparable to the Feulgen Technique.

Although we expect to find increased amounts of nucleic acid in cancer cells, any verification of this concept in terms of measurements, quantitative or semi-quantitative, might give us a better understanding of the processes active in the particular biological system under study. Dr. Sandritter points out that larger series of cases should be evaluated, which might require more efficient measuring procedures than he has access to. The evaluation of cytophotometric data still requires too many calculation steps to make this procedure suitable for the routine investigation of clinical cytological smears.

CLOSING REMARKS

WALTER SANDRITTER, Frankfurt am Main, Germany:

Further measurements are currently being made. We have also considered an improvement of the methods employed in routine work (see Tolles). We are at present using a relatively larger opening (Plug-measurements) in measuring the relative nucleic acid content, and also we have found that the size of the nucleus may be better estimated with the help of a "ring-system" in the ocular.

ADVANTAGES AND DISADVANTAGES OF THE FEULGEN REACTION AND HISTOPHOTOMETRIC TECHNIQUE AS APPLIED TO CERVICAL SMEARS

PIERRE HAOUR and CLAUDE CONTI
Lyon, France

Feulgen and Rosenbeck (1) proposed a specific reaction for D. N. A. in cell nuclei. This staining procedure has been used extensively for tissues and different histophotometric techniques and has been reported effective for the quantitative measurement of nucleic acid. The procedure can also be applied to cytological material.

We have been studying cervical smears from patients selected from a group having routine cytological procedures. Values were obtained for optical density of nuclei and nuclear diameter by measurement with the Lison histophotometer. Results are illustrated in a diagram (Fig. 1) based upon the values found in measuring the D. N. A. ratio of nuclei of buccal cells. These values appeared to be double the D. N. A. content of the spermatozoid, therefore representing a diploid value (2N). Results obtained on this basis may be easily controlled by other laboratories, regardless of the apparatus used.

The Feulgen reaction produces a red color in the nuclei; the chromatin structure appears more distinct than with the Papanicolaou stain. The color reaction is proportionate to the D. N. A. content of nuclei, which in tissues was found to be higher in the nuclei of cancer cells than in normal cells. Histophotometry could, therefore, make it possible to measure the difference between normal and malignant cells. This difference appears when histophotometry is applied to cervical smears (see diagram). Of 172 cases studied by this method, 138 out of 141 cases with negative biopsy had histophotometric values in the lower range (under 6N): 3 cases had negative biopsy but showed repeatedly high values of D. N. A. and had several Papanicolaou Class IV evaluations; 31 cases with positive biopsy all showed values in the highest range (over 6N).

As compared with the Papanicolaou technique, histophotometry and the Feulgen reaction can reduce the number of doubtful and false-positive cases in routine diagnosis. In 15 cases (14 Class III and one false-positive) histophotometry yielded results corresponding to pathology.

Dyskaryotic cells were also studied in cases of Trichomonas vaginitis; D. N. A. values were found to be higher than in normal nuclei, but never reached the figure of malignant cells.

With Feulgen reaction, the staining technique and the quantitative measurement need special care and may appear time-consuming. Of course, it should not be considered as a routine procedure, but it can be successfully applied in controlling the doubtful and false diagnoses of the Papanicolaou technique.

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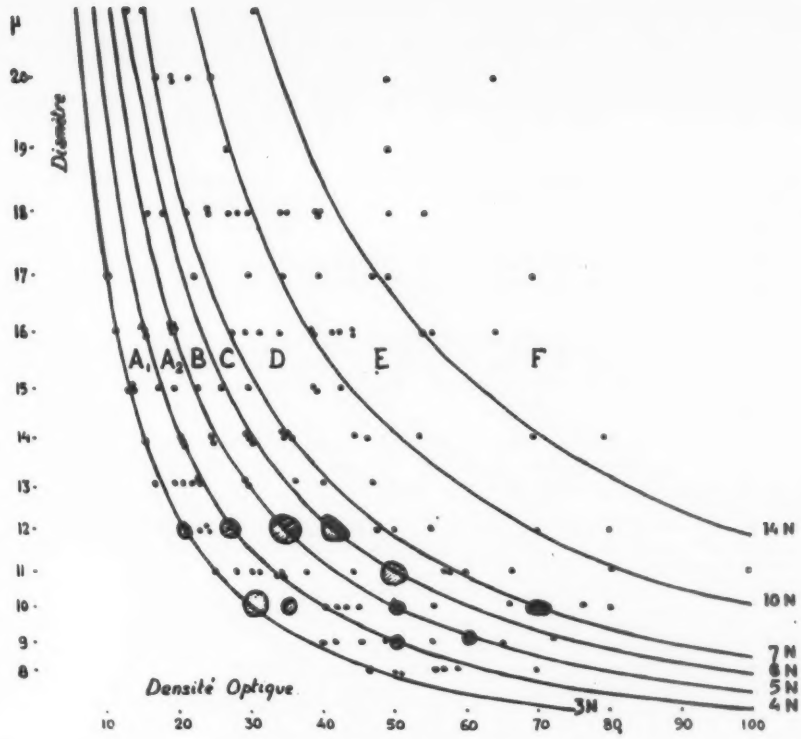

Fig. 1. Diagram showing values of D.N.A. in cervical cells. Curves are based upon the values obtained from measuring by histophotometry the 2N diploid ratio of buccal cells. The curve 6N represents the limit above which malignant cells are found; under this limit cells from cases with negative biopsy are encountered.

DISCUSSION

GUNTER F. BAHR, Stockholm, Sweden:

Any approach to support the subjective judgment of a smear by measurements--although time-consuming and elaborate, but objective--is very welcome. This holds especially for doubtful cases, for which Haour and Conti recommended the histophotometric technique of Lison.

Such cases are, however, doubtful because their morphology and staining properties do not lend themselves to make a clear diagnostic decision. Their Feulgen - histophotometric values will almost certainly be found in a range close to the critical borderline of negative or positive in the proposed system. I assume that the validity and reliability of a borderline of 6N (taking the Feulgen value for buccal epithelial cells for 2N) will be questioned by a number of laboratories.

WALTER SANDRITTER, Frankfurt a. M., Germany:

I would like to point out that the intensity of the Feulgen reaction is dependent on various, not always controllable, factors. The pertinent question here is: how can we overcome these difficulties? The application of a basic stain such as Gallocyanin-chromalume, has, in my opinion, definite advantages in this respect. The increased RNA content of the carcinoma cell nucleus can be clearly demonstrated, which serves as a distinguishing feature for the respective recognition of carcinoma and normal cells.

R. VOKAER, Brussels, Belgium:

Since 1951 we have been using (1, 2, 3, 4, 5) the histophotometric technique employed by Haour and Conti to determine the desoxyribonucleic acid (DNA) content of cell nuclei in vaginal smears. We cannot but insist once more on the interest of this method which, as used by Lison (6), permits the estimation of the DNA content of individual nuclei in smears stained with the Feulgen reaction. The method postulates that the intensity of the Feulgen stain is directly proportional to the amount of DNA present. This very important point has been fully discussed by several authors (7, 8, 9, 10) and is now generally regarded as satisfactorily proved.

Applying this optical method, we have studied the variations of the DNA content of the nuclei of vaginal cells not only during several estrous cycles but also in smears for the identification of malignant cells.

In July, 1957, at the First International Congress of Cytology held in Brussels, we announced the first results obtained in cases of smears containing malignant cells of cervical as well as endometrial origin (11).

The graphic illustration of the DNA ratio of cells makes it possible to build histograms, the reading of which immediately indicates the presence of a certain number of pathological nuclei. Values, double and quadruple the average content in DNA, are registered, revealing the presence of neoplastic polyploid nuclei.

Of course these histophotometric measures require an expensive and complicated apparatus and they are not yet in general use. However, the use of this investigation procedure should be generalized, as it can be of great help to the cytologist.

We thus appreciate very much the fact that Haour and Conti in their studies confirm our own results published a year ago, and we do hope to be followed by many authors in this way.

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CLOSING REMARKS

PIERRE HAOUR, Lyon, France:

Answering Bahr, I would say that histophotometry applied to Feulgen reaction is not an absolute method to identify cancer cells, and that the system of quantitative analysis we proposed was considered only practical to represent our results and to be able to compare them with those from other laboratories interested in the same technique. Therefore, in our hands histophotometry has been quite helpful in reducing an appreciable number of errors and especially in doubtful cases of the routine technique. Our actual experience with 243 cases analyzed shows that among 42 doubtful false positive and false negative cases the Feulgen reaction gave correct results according to the clinical and histological findings in 30 cases. Doubtful cases, which represent the largest group, all appeared clearly negative or positive with histophotometry: D. N. A. values obtained were always found in the lower or the higher range, never close to the 6N limit. Cases with values approaching or located on this limit were never doubtful with the Papanicolaou technique. Twelve cases analyzed had results which did not correspond to histology and still remained false positive or false negative. We must point out that to avoid false negative it is necessary to measure more than one smear in a single case.

The result of the histophotometric technique should therefore be considered as complementary to the Papanicolaou routine diagnosis.

I am not of the opinion of Sandritter that the intensity of Feulgen reactions depends on so many varied factors. The Fuchsin dye should be chosen carefully and tried before any histophotometric measure on several smears of the same material; the different durations of the coloration must be carefully respected and slides must be analyzed not later than eight days afterward.

Provided these precautions are carefully observed, Feulgen reaction is quite reliable for quantitative analysis.

I have no experience either with the Gallocyanin chrome alum stain, but I was very interested in the results that Sandritter obtained by this method.

I appreciate that Vokaer could confirm the advantages of the histophotometric technique, reporting on his long experience with the method.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

Symposium C

CYTOLOGICAL EFFECTS OF ADMINISTERED ESTROGENS (EXOGENOUS ESTROGENS)

METABOLISM OF ESTROGENS

ERNST JÜRGEN PLOTZ
Chicago, Illinois, U.S.A.

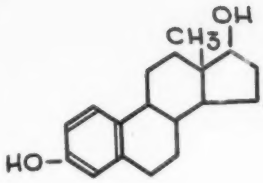
The available evidence indicates that estradiol-17-β (Figure 1) is the estrogenic hormone produced by the ovaries and other endocrine glands. This compound is the most potent, naturally occurring estrogen and is excreted in small amounts in the urine, together with estrone and much larger amounts of estradiol (Figure 1). Repeated experiments (1,2) have shown that estradiol and estrone are interconvertible in the human organism. This transformation is not dependent on the presence of the ovaries, since it occurs in both males and females. As shown by Ryan and Engel (3) in in vitro experiments, a variety of normal and abnormal human tissues have the capacity to interconvert estrone and estradiol (placenta, testes, liver, breasts during pregnancy, mammary fibroadenoma and adenocarcinoma, uterine fibromyomas, hyperplastic endometrium, hyperplastic adrenals). Levitz et al (4) studied the interconversion of C^{14}-labeled estrone and estradiol in perfused human placenta. Reproducible ratios of estrone to estradiol were observed in the perfusate. Furthermore, human feces contain an enzyme which reduces estrone to estradiol as shown by Stimmel (5).

Both compounds, estradiol and estrone, can be converted into estriol (Figure 1) by human subjects (6, 7). When estradiol-17-β was administered to women, a definite increase in the urinary output of estradiol, estrone and estriol was observed. The injection of estrone also resulted in an increase of all three compounds, although the total percentage recovery was somewhat smaller as compared with that found after the administration of estradiol. Beer and Gallagher (8) measured estrone and estriol in the urine of non-pregnant women following the administration of known amounts of estrone-16-C^{14} and estradiol-16-C^{14}. Estrone accounted for 8-10% and estriol from 11-31% of the total urinary activity. The amounts of these metabolites appeared to be similar for either precursor. Smith and Smith (7) have claimed that the conversion of estradiol and estrone to estriol is favored in the presence of adequate progesterone, whereas this conversion is minimal when the endogenous production of progesterone is deficient. However, experiments carried out by Pearlman et al (9) using deuteriated estrone failed to support the view of Smith and Smith. They injected isotopic estrone into two pregnant women and determined the urinary deuteriated estriol. The percentage of the injected hormone recovered from estriol was almost identical when compared with that obtained in men. The conversion of estradiol (and estrone) to estriol seems to be irreversible, since only unchanged estriol is recovered in the urine, the amount being around 30% regardless of the administered dosage (6).

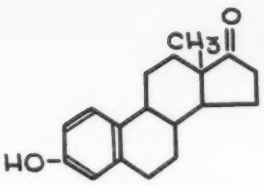
Very recently, Marrian et al (10) discovered a new estrogen, 16-α-hydroxyestrone (Figure 1), in the urine of pregnant women. They suggested that this compound may be formed from estrone and be the metabolic intermediate in the conversion of estrone to estriol. Stimmel (11) was likewise successful in isolating this new estrogen from urine during pregnancy and found the following estrogen titers in a 24-hour urine specimen: estriol 6.35 mg.; 16-epiestriol 0.116 mg.; 16-α-hydroxyestrone 0.528 mg.; estrone 0.292 mg.; and estradiol-17- 0.095 mg.

In addition to these estrogens, the urine of women contains material which remains non-estrogenic after acidification and heating, but is rendered estrogenic when powdered zinc is added to the acidified urine prior to heating. These unidentified compounds have been called estrogen oxidation products by Smith and Smith (7). But even the inclusion of these oxidation products together with the known estrogens did not substantially increase the urinary recovery of metabolites (about 16%) after the administration of estradiol.

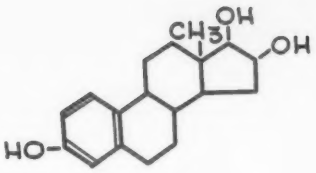
When isotopically labeled precursors became available, further advances concerning the metabolism of estrogens were made. Beer and Gallagher (8) demonstrated that about 65% of the radioactivity derived from radioactive estrone or estradiol was recovered in the urine of non-pregnant women. However, the known urinary estrogens accounted only for half of the total radio-



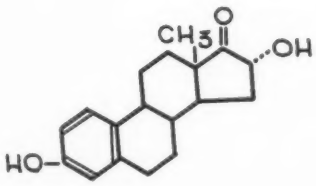
ESTRADIOL-17(β)



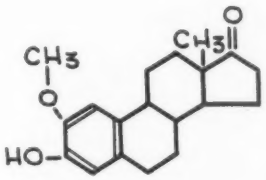
ESTRONE



ESTRIOL



16 (α)-HYDROXYESTRONE



2-METHOXYESTRONE

Fig. 1

INTERCONVERSION OF HORMONES

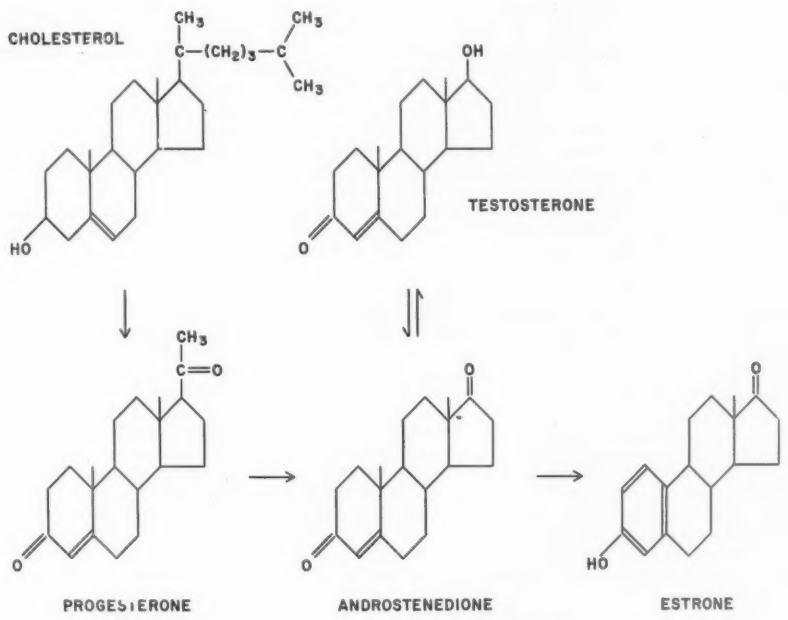


Fig. 2

activity in the urine. There is strong evidence that a new estrogen, 2-methoxyestrone (Figure 1), which was isolated by Kraychy and Gallagher (12) in human urine, represents another metabolite of estradiol. Engel et al (13) injected estradiol-17-β-16-C^{14} into a post-menopausal patient and subsequently recovered radioactive 2-methoxyestrone in the urine. In their experiment, this compound was by no means a minor metabolite, since it accounted for roughly 8% of the administered radioactivity. There seems to be no doubt that other hitherto unknown metabolites of estradiol will be discovered in the near future.

The liver plays an important role in the intermediate metabolism of estrogens. The in vitro destruction of estrone or estradiol both by liver slices or minces and by perfusion through the liver has been demonstrated (14, 15, 16). The nature of the inactive products remains obscure, although many of these products seem not to be phenolic as shown by Lieberman (17). In vivo experiments with estrone-16-C^{14} indicate that the D-ring of the steroid molecule may be destroyed during its metabolism since radioactive CO_2 was recovered in the expired air (18). The exact nature of the enzyme systems involved in the degradation process of estrogens within the liver is not known, but oxidative systems appear to be involved in these transformations (19). Indirect evidence for the ability of the liver to degrade estrogens to inactive products is demonstrated by the observation that excessive amounts of estrogens may be excreted in individuals with hepatic disease (20).

Despite the fact that estrogens cannot be recovered from the liver (due to rapid inactivation processes), a considerable degree of estrogenic activity is exhibited by bile after the injection of estrogens (21). Following the injection of radioactive estrone or estradiol about 7-11% of the administered radioactivity was excreted into the feces (8). These observations suggest that estrogens excreted by the bile into the gastro-intestinal tract may subsequently undergo an enterohepatic circulation (22). This view might explain the relatively delayed excretion of estrogen metabolites after intravenous injection.

Until very recently virtually nothing was known concerning the mechanism of the biosynthesis of estrogens. In 1953, Werthessen et al (23) perfused sow ovaries with C^{14}-labeled acetate and isolated radioactive cholesterol, estrone and estradiol from the perfusate. When human ovarian tissue slices were incubated with C^{14}-acetate, radioactive estrone was recovered in the supernatant. Similarly designed experiments in our laboratory revealed that human placental tissue is capable of synthesizing estrone from acetate (Table 1). When C^{14}-1-acetate was administered to a pregnant woman scheduled for therapeutic abortion because of cancer of the cervix, we recovered pure estrone in the urine which was radioactive (Table 2).

Since cholesterol is produced from acetate simultaneously with the estrogenic hormone, one could presume that these hormones are derived from this cholesterol. However, Heard et al (24) were not able to find any radioactivity in urinary estrone when labeled cholesterol was administered to a pregnant mare, whereas acetate served as a precursor of estrone in this species. The authors considered the direct synthesis of estrone from acetate (independent of cholesterol) as the major pathway of estrogen synthesis in vivo. We were unsuccessful in our first attempts to recover labeled estriol from the urine of two pregnant women who received cholesterol labeled with tritium (25). These findings seemed to support the observations of Heard et al. However, relatively small amounts of tagged cholesterol were used in our initial experiments. Therefore, we administered a total of 87.4 μc of C^{14}-4-cholesterol over a period of six days to a pregnant patient, during which time all the urine was collected and processed as a single pool (26). Using large amounts of carrier radiochemically, pure estrone was recovered and found to be radioactive (Table 3).

Since our experiment demonstrated that estrogens can be synthesized from cholesterol in human pregnancy, it is no longer necessary to assume a pathway of estrogen biosynthesis independent of cholesterol in vivo, although such a pathway may exist.

It had been suggested for a long time that androgens of the type of testosterone and 4-androstenedione may serve as precursors of estrogens (See E. J. Plotz, ACTA CYTOLOGICA 1: 71, 1958). When human ovarian tissue obtained from pre- and postmenopausal women was incubated with labeled testosterone, radioactive estrogens were isolated by carrier dilution technique (27, 28). The following study was designed to investigate whether such a conversion is in actual operation during human pregnancy. Following the administration of C^{14}-4-testosterone, a total of 0.8% of the administered dose was recovered in the crude phenolic fraction of the urinary steroids. Using large amounts of carrier, pure estrone was identified. As seen in Table 4, this estrone was radioactive. Our data lend support to the conclusion that the metabolic pathway cholesterol-testosterone (Δ^4-androstenedione-estrone) is one of the routes of estrogen biosynthesis in human pregnancy. In vitro experiments by A. Meyer (29) revealed that the major site of the conversion of androgens to estrogens may be the placenta. When Δ^4-androstenedione was incubated with human placenta, its conversion to estrone was demonstrated. Meyer's experiments strongly suggested that the conversion proceeds via such $C_{(19)}$-hydroxylated intermediates as 19-hydroxy-Δ^4-androstenedione. The in vivo conversion of testosterone to estrone was also demonstrated in the pregnant mare (30).

In all cases of androgen-estrogen conversion, however, the yield is of the order of 1%, which is relatively too low to account for the large amounts of estrogens excreted in pregnancy. Therefore, other pathways of estrogen biosynthesis may be in operation in human pregnancy.

Since it is known from the results of several in vivo and in vitro experiments that progesterone can be converted into androgens (31, 32) and — as outlined above — androgens can be converted into estrogens, one could assume that progesterone may serve as a precursor of estrogens. So far we have not been able to demonstrate a progesterone-estrone conversion in normal pregnancies. However, when 37.81μc of C^{14}-4-progesterone was injected intra-muscularly into a patient with metastatic choriocarcinoma, 0.434μc (=1.1%) of the administered dose was found in the crude phenolic fraction. After adding a carrier pure estrone was isolated and radioassayed. The counting data are shown in Table 5. The specific activity of this material remained constant when its derivatives were formed, indicating radiochemical purity.

A tentative scheme of one of the pathways of hormonal interconversion leading to estrogens is shown in Figure 2.

Table 1. Specific activities of estrogens (isolated by carrier method) after incubation of human placental slices with C^{14}-1-acetate.

Compound	Mg. Counted	Free Steroid DPM/Mg. **
Estrone benzoate	91	0.93
Estrone benzoate*	67.8	0.88
Estradiol monobenzoate	45	0.75

*After hydrolysis, then reformation and crystallization.
**Corrected for quenching.

Table 2. Specific activities of estrogens (isolated by carrier method) following the administration of C^{14}-1-acetate (human pregnancy).

Compound	Mg. Counted	Free Steroid DPM/Mg. **
Estrone acetate	49.2	1.34
Estrone acetate*	16.5	1.44
Estradiol diacetate	56.3	1.61
Estradiol diacetate*	33.6	1.44

*After hydrolysis, then reformation and crystallization.
**Corrected for quenching.

Table 3. Specific activities of C^{14}-estrone (isolated by carrier method) following the administration of C^{14}-4-cholesterol (human pregnancy).

Compound	Mg. Counted	Free Steroid DPM/Mg. **
Estrone acetate	117.4	15.9
Estrone acetate*	96.1	16.0
Estrone benzoate	84.7	14.7
Estrone benzoate*	77.0	14.5
Estrone benzoate*	68.9	15.3

*After hydrolysis, then reformation and crystallization.
**Corrected for quenching.

Table 4. Specific activities of C^{14}-estrone (isolated by carrier method) following the administration of C^{14}-4-testosterone (human pregnancy).

Compound	Mg. Counted	Free Steroid DPM/Mg. **
Estrone acetate	39.1	29.9
Estrone acetate*	17.8	30.6
Estrone**	6.7	28.9

*After hydrolysis, then reformation and crystallization.
**After hydrolysis.
***Corrected for quenching.

Table 5. Specific activities of C^{14}-estrone (isolated by carrier method) following the administration of C^{14}-4-progesterone (chorionepithelioma).

Compound	Mg. Counted	Free Steroid DPM/Mg. **
Estrone benzoate	260.2	2.93
Estrone (1st Cryst.)	180.1	2.91
Estrone (2nd Cryst.)	175.5	2.93
Estrone acetate	172.1	3.33

*Corrected for quenching.

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DISCUSSION

KARL JUNKMANN, West-Berlin, Germany:

The excellent compilation of published data, supplemented by the author's own experience, gives a clearcut picture of our present knowledge of the biosynthesis and the metabolism of estrogens. Furthermore, it shows the gaps in our knowledge and will certainly fructify future research in the field. The cautious and critical evaluation of the different experimental results is to be appreciated. It appears that a number of hitherto unknown metabolites of estrogens still has to be anticipated.

Experiments have revealed some fundamental pathways by which biosynthesis of estrogens may proceed in the ovary as well as in the placenta. One has the impression that estrogens are formed from acetate relatively easily, while the yields of estrogens cholesterol via pregnenolone, progesterone, 17-α-hydroxyprogesterone, androstendione, testosterone, and 19-hydroxytestosterone (a biosynthetic pathway known to occur in the adrenal cortex) are so low that one is tempted to consider this as a side-route. However, one can imagine that cholesterol produced in vivo may be transformed into estrogens in the ovaries and the placenta with much higher yields than cholesterol added to living organs. The same would hold true also for progesterone and testosterone.

The assumption that acetate easily penetrates into hormone-producing cells while cholesterol or other steroidal precursors will do this only with difficulty should be proven experimentally. Experiments with formed, isolated enzyme systems from hormone-producing cells should be undertaken, or attempts should be made to localize labeled precursors in the cells by histological methods.

If it would be possible to confirm the assumption mentioned above, the biosynthesis of estrogens from cholesterol via progesterone and testosterone would attain much more importance.

CLOSING REMARKS

ERNST JÜRGEN PLOTZ, Chicago, Illinois, U. S. A.

I wish to thank Junkmann for his discussion and kind remarks. I certainly agree with his statement that our present knowledge of the biosynthesis and metabolism of estrogenic hormones will be enlarged in the near future. It is entirely conceivable that other pathways will be discovered which may be of greater importance and significance than those known at the present time.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

METHODS, OTHER THAN EXFOLIATIVE CYTOLOGY, FOR DETERMINING THE EFFECT OF ADMINISTERED ESTROGENS

JACQUES FERIN
Louvain, Belgium

In other words, how can the following questions be answered with a method other than exfoliative cytology:

1) Is this compound estrogenic in human beings?
2) If yes, is it a strong or weak estrogen?
3) How long does the estrogenic effect last?

Generally, in order to appreciate, qualitatively and semi-quantitatively, the effects of the administration of an estrogen in women, it is necessary to select subjects in whom the endogenous production of estrogens is and remains practically negligible, as in certain cases of gonadal agenesis (Turner's syndrome) or after surgical castration. One can add certain secondary (precocious menopause, for example) and postmenopausal amenorrheas to this group; in these cases one can, in fact, consider as very improbable the eventuality of an appreciable spontaneous recovery of the endogenous secretion of estrogens.

On the other hand, after castration by X-Ray or radium, ovarian production of estrogens is not always completely suppressed, as it can reappear after a certain time. It should not be forgotten either that the genital tract, when once subjected to irradiation, loses some of its receptivity.

One should see that the subjects so selected fulfill certain conditions relative to the intermediary metabolism of both natural and artificial estrogens: the hepatic, intestinal and renal functions must be normal. Obesity may also be considered an interfering factor; it is known from some observations that fatty tissue can store estrogens.

It is, moreover, necessary that the endocrine system should not present any gross anomalies apart from estrogen deficiency. One knows, actually, that an increased activity of androgens may interfere with the estrogenic effects on the target organs. Thus, a given dose of estrogen, effective in the normal subject, will remain without effect in the adreno-genital subject.

It is only by acting strictly in the selection of subjects that one can hope to reduce the importance of individual variations in the reactions of the organism to estrogenic stimulation. Nevertheless, these individual variations remain an important problem. The investigator who wants to study the relative effectiveness of several estrogens cannot generally acquire a sufficiently large number of adequate subjects which allows statistical evaluation of the results. Therefore, he finds himself forced to administer the different substances successively to a few selected women.

Concerning the response of the organism to estrogenic stimulation, one can consider two factors: a) the intensity of the estrogenic effect and b) the duration of that estrogenic effect.

Each of the methods utilized can give us simultaneous information on these two factors, or only on one of the two.

As the principal target organ of estrogens the uterus should be studied first. In an individual used for study, the uterus must be free of any infections, neoplastic or cicatricial lesion. The uterus must be sensitized to estrogenic action: the infantile uterus, which has never undergone estrogenic stimulation, just like the atrophic uterus which has been deprived of it for a long time,

does not react perfectly to the first stimulus, even if it is very strong. In order that the organ may acquire its maximal reactivity, a previous treatment with estrogen of variable duration is needed. Sometimes this is no longer possible because of irreversible changes. This responsiveness of the uterus does not seem to be altered with age.

One can study different reactions of the uterus:

1. The effect of estrogenic treatment on uterine muscle and vascularity.
2. The effect of estrogenic treatment on the muco-secretory activity of the endocervical glands.
3. The effect of estrogenic treatment on the endometrium.

1. Uterine Muscle Vascularity and Motility

Estrogenic stimulation produces a turgescence of the uterus due to the augmentation of interstitial fluid, congestion of the organ and hypertrophy of the myometrial cells. This results in an elongation of the uterine cavity by several centimeters, easily measured by hysterometry. When the estrogenic action ceases, the uterine volume size slowly regresses again and the measurements of its length show a decrease (1, 2).

One can appreciate to a certain extent the duration of the estrogenic effect by this method, but it is not possible to evaluate its intensity.

It is necessary to mention here that one could evaluate the modifications of the uterine circulation and vasodilating action of estrogens by means of the intra-uterine introduction of a special apparatus equipped with a thermo-electric couple (3, 4).

One could equally well study, by the intra-uterine balloon method, changes caused by estrogens in myometrial motility (5).

2. The Endocervical Glands

Estrogenic stimulation, if quantitatively sufficient, releases in the cervix, after one to four days, the secretion of a translucent, alkaline, thread-like mucus (6, 7), which is easily invaded by the spermatozoa, and is particularly favorable to their survival. One can determine the minimal quantity of an estrogen which releases this characteristic secretion.

For a given subject, and in certain limits, the degree of "spinnbarkeit" and the quantity of mucus seems proportional to the estrogenic dose (personal observation) (8). However, it would be necessary to determine the possible interaction of the sympathetic nervous system, sexual response and orgasm.

The muco-secretory activity does not decrease with time; its persistence measures the duration of estrogenic effect.

The thread-like mucus, after being dried on a slide, crystallizes characteristically; this is the Fern test. One can determine the minimal quantity of an estrogen which releases the appearance of these arborizations (9).

3. The Endometrium

The endometrial reactions can be controlled most precisely and accurately by the histological study of a biopsy with Novak's curette, taken from the fundus where the mucosa presents maximal receptivity to hormonal stimuli. The biopsies will be done by the same person with the same technique. It is relatively easy to evaluate, for a given subject, the threshold dose of an estrogen, which, administered during a fixed period of 14 days, for example, determines the beginning of endometrial growth (10).

It is necessary to define what is meant by "beginning of endometrial growth." After administration of a subthreshold dose, the curette retrieves only a small piece of endometrium, with some connective tissue and a few narrow glandular tubes; there is no mitosis, the nuclei are usually compact. After administration of a threshold dose, the biopsy is distinctly larger, the glandular tubes of greater diameter and show mitosis; the nuclei of the cellular chorion are more often larger with dispersed chromatin.

It is very important to note, as we have already observed, that this threshold dose in a given subject remains practically constant for several years. An ovariectomized woman has been observed during nearly 20 years, from this point of view.

To evaluate the relative importance of supra-threshold doses administered over a period of two to three weeks, for example, is a more delicate problem; parallel with the increase of estrogenic dose, one observes a tendency towards an increase in the diameter of glandular tubes and the thickness of their walls, while the pseudo-stratification of the nuclei is accentuated (1, 2, 11).

The development of a glandular-cystic hyperplasia of the endometrium indicates a marked and especially prolonged estrogenic stimulation of at least six weeks' duration.

When the estrogenic dose is prolonged, but of feeble intensity, one observes a particular picture characterized, notably, by the following criteria:

1) thinness of the functional endometrial layer,
2) scarcity of mitotic figures,
3) variability of glandular diameter, and
4) dense, compact aspect of the cellular chorion.

It is difficult to present a really demonstrative picture of these different aspects; it is necessary to be able to study both the biopsy as a whole and its detail, and there is no doubt that, to a certain extent, a subjective factor of appreciation intervenes.

In order to study the endometrial effect of estrogens, one can equally rely on withdrawal bleeding (12, 13, 14). The appearance of such hemorrhage actually supposes that the endometrium has undergone a previously sufficient growth.

One can determine the minimal dose capable of provoking this withdrawal bleeding (10, 13, 15, 16).

This method offers a certain practical advantage: gynecological examination, and especially endo-uterine biopsy, can be avoided. From this fact, it will certainly be easier to obtain the cooperation of patients.

However, it is certain that withdrawal bleeding does not always occur when the endometrial growth is adequate (1, 10).

The latent period, i.e., "lag-time" between the end of the estrogenic treatment and the beginning of withdrawal bleeding, constitutes a particularly precise measure of the duration of the estrogenic effect (17), as I have outlined in a previous communication.

Estrogens assure not only a stimulating action on the uterus and the whole genital tract, but they influence more or less all the systems of the body. Certain of these actions are particularly well known and have served as a basis for methods of estimating the effects of estrogenic treatments.

One knows that vasomotor disorders of castration disappear under the influence of estrogens. However, these disorders are influenced to such a point by other factors, notably psychic, that it is particularly hazardous to rely on the disappearance of hot flushes, for example, for the study of the effect of estrogenic treatment (18). Whatever might be the case, we personally think that this method can be valuable in certain selected subjects.

The increased urinary excretion of gonadotrophins in castrated and post-menopausal women disappears under the influence of adequate estrogen doses; the urinary level of gonadotrophins returns to normal. One can study the relative potency of various estrogens, from this point of view.

However, it is a complicated and uncertain method for the following reasons: decrease of the gonadotrophin level is only produced, generally, after a latent period which is very variable from one subject to another; the fall of the urinary level, necessary in order that the reaction be considered positive, is fixed arbitrarily; the determination itself is time-consuming and requires a large number of animals; the urinary level can vary considerably from one woman to another, and in the same woman from day to day before any treatment has been given (21, 22).

In conclusion, it is necessary to emphasize the fact, of cardinal importance, that only the typical endometrial proliferation and endocervical threadlike mucus secretion are <u>specifically estrogenic</u> effects.

The disappearance of vasomotor disorders and lowering of the urinary hypergonadotrophic level can be obtained by the administration of <u>non-estrogenic</u> substances.

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Fig. 1. Endometrial biopsy after subthreshold dose.

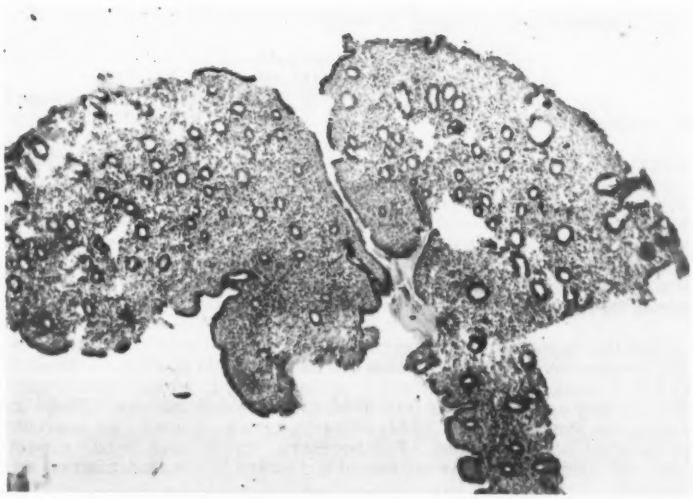

Fig. 2. Endometrial biopsy after threshold dose.

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DISCUSSION

EMMERICH VON HAAM, Columbus, Ohio, U.S.A.:

The author recommends two practical and fairly accurate methods, other than exfoliative cytology, to determine the effect of administering estrogens. These are the Fern test of cervical mucus and the recognition of typical endometrial proliferation as demonstrated by endometrial biopsy. He further emphasizes the appearance of withdrawal bleeding and particularly the time period between the end of the estrogen treatment and the beginning of the bleeding as a particularly precise measure for the duration of the estrogen effect. In my capacity as consultant to the endocrine clinic, I have been interested in this subject for many years, particularly during the period when we tried to replace the expensive biological estrogens by the less expensive synthetic stilbesterol and similar substances. We used surgical castrates exclusively and tried to determine the effectiveness of the administered estrogen by laboratory analysis for urinary gonadotropin in correlation to the disappearance of the known vasomotor disorders. This laboratory procedure proved quite expensive, necessitating the maintenance of a carefully controlled mouse colony, but gave scientifically good results. It also demonstrated that the sensitivity of the patients' vasomotor systems to blood gonadotropins varied a great deal for different individuals so that no standard correlation could be obtained. We agree that other steroids can produce similar results and therefore have to be considered or very carefully excluded. Endometrial biopsy is not only a procedure against which some patients will object but it is difficult to repeat it at short intervals since the healing process evoked by one endometrial biopsy interferes with the clear-cut endometrial picture of a subsequent biopsy. The Fern test has proven an excellent way of recognizing an estrogenic response, provided the test was negative before administration of the estrogen and provided that no quantitative evaluations are desirable. In our experience it is the best method to be recommended as a simple office procedure for the practitioner who would like to obtain some type of objective evidence of the effectiveness of his estrogen therapy.

CLOSING REMARKS

JACQUES FERIN, Louvain, Belgium:

I agree with Von Haam in considering the Fern test (or simply the presence of clear, threadlike mucus at the external cervical os) as an excellent index of estrogenic effect, which is especially interesting from a practical standpoint.

The other tests that we have described, namely, the histological study of endometrial reactions, are primarily of scientific interest. Rarely, actually, are patients sufficiently cooperative in agreeing to be submitted to repeated endometrial biopsies! We do not think that the traumatisms produced by these repeated biopsies might modify the endometrial picture.

We have had the opportunity to observe three patients over a period of 19 years, 9 years, and 5 years, respectively. A biopsy was taken at the end of each of the artificial cycles which varied from 2 to 5 weeks. These different cycles ensued, without interruption, at intervals of 6 to 15 days. The biopsy specimen was procured with a Novak curette without using suction, while slightly scraping the fundus uteri. Proceeding in such a manner, we have never found any healing process in the endometrial pieces. Furthermore, as outlined in our report, we have neither observed any variation during the course of the years in the endometrial receptivity to administered estrogens.

DO ADMINISTERED ESTROGENS STIMULATE GROWTH AND MATURATION OF THE EPITHELIUM DIRECTLY OR INDIRECTLY THROUGH STIMULATION OF THE NERVOUS SYSTEM AND ENZYME SYSTEM?

JEAN de BRUX
Paris, France

Since Brown-Sequard's demonstration on himself (1) of the endocrine role of the testes, the discovery of the natural and—later—of the synthetic hormones has increasingly interested physiologists in these substances which act on tissue growth and the regulation of the functions.

Various theories as to the mechanism of their action, while perhaps a priori antagonistic, are nevertheless indissociable. Some hold that the hormones act by enzymatic action. Others believe that they modify the autonomic nervous system of the receptor organ, and that their action is thus neuro-hormonal. It is probable that both approaches to the problem have some part of truth. Before discussing them, we should note the following:

(1) The existence of a specific sensitivity of an organ, and even of certain zones of such organs, to one or another hormone.

(2) The notion of such sensitivity varying from one individual to another, due to:

(3) A threshold of action, having:
   (a) a minimum limit, corresponding to the minimum hormonal dose required to obtain a given effect, and
   (b) a maximum limit, above which the effects may be either arrested or reversed (particularly in the connective tissue).

I. ENZYMATIC THEORY

The hormones act on the enzymes, either:

(1) by modifying their concentration in the tissues,
(2) by acting as a constituent of the enzymatic complex itself, or
(3) by activating or inhibiting the enzymatic systems.

However, it is difficult to determine which of these categories of action to attribute to the estrogens, because research up to now has been "in vitro" and for this reason could lead to erroneous conclusions from an "in vivo" standpoint.

We know that in the organism the estrogens are present in two forms, free and combined; in both forms they act on the growth of the tissues of sex organs.

The estrogens may be an integral part of an enzyme system:

(1) by combination with a particular protein:

estrogens + specific protein = enzyme X (not yet identified), acting directly on the tissues;

(2) by reacting, as substratum, with an enzyme:

estrogens + enzyme = active enzymatic complex.
(substratum)

On this subject the works of W. H. Fishman (2), S. L. Cohen (3), Kerr, Campbell and Levy (4) and those of Buehler (5) are noteworthy. For lack of space, we shall give here only the conclusions of these works:

(a) The estrogens raise the β-glucuronidase level of the genital tract. According to Fishman (2), it acts as a catalyzing enzyme in a complex system of synthesis of the glucuronates of estradiol. In this connection, S. L. Cohen (3) has demonstrated that ovariectomy determines a marked reduction of the glucuronidase level in the uterus, whereas in the vagina this reduction is much less marked. This difference may be explained by the extreme sensitivity of the vaginal epithelium as compared with that of the uterus, and also by the formation of glucuronidase by the leukocytes, which become more numerous with the cessation of the estrogenic action. The diminution of this enzyme is strictly limited to the genital tract.

(b) The estrogens reduce esterase formation at the uterine and vaginal levels.

(c) The estrogens increase considerably the alkaline phosphatase. According to Kerr (4), the alkaline phosphatase curve follows very closely that of the glucuronidase.

(d) According to McShan and Meyer (6), the estrogens inhibit the succinoxidase by inhibition of cytochrome oxidase.

These purely biochemical notions find their confirmation in tissue cultures. Gailiard (7) (1942) was the first to demonstrate the effect of the sex hormones on uterine explants. In the presence of estrogens, these explants continue to develop, whereas the controls without estrogens deteriorate rapidly. Furthermore, Wolff and Haffen (8) (1952) showed the role of the estrogens in the differentiation of the gonads in chickens. A rudimentary testicle, cultivated in a medium containing estrogens, produces a structure of ovarian type, instead of forming spermatic tubes as in the case of the control cultivated in a normal medium. However, these facts only show the growth-determining effect of the estrogens on certain tissues having a specific receptivity to these hormones. It seems unlikely that the estrogens act by simple contact with the cervico-vaginal and endometrial epithelium. The most probably hypothesis, therefore, would be that they form a part of the enzymatic complexes; this, however, is yet to be proved.

II. NERVOUS SYSTEM THEORY

A. Anatomy of the sympathetic nerve endings:

The nerve endings in the chorion and the mucous membrane are revealed by careful fixation and specific colorations:

(1) Champy's method (osmium potassium iodide)

(2) Lombart-Jabonero's method, as modified by Isidor.

By means of these techniques, Coujard (9) has shown the existence, beneath the vaginal mucous membrane, of nerve cells with isolated or, more rarely, grouped Nissl bodies; spreading out from these elements is found an extensive network of terminal fibers with interstitial sympathetic cells.

Champy (10) demonstrated in 1922 that the hormones determine localized growth phenomena in definitely limited regions of the organism, to the exclusion of other regions. These "sensitive zones" are complex areas in which all the tissues react at the same time, the sensitivity being pluri-tissular. There is nothing in the aspect of the tissues, before the hormonal action, which reveals, a priori, their sensitivity; this is revealed only by the hormonal action itself.

In the genital tract and in the breast the predominating phenomena are vasomotor and are commanded by the sympathetic nervous system. The latter, however, is under the control of the hormonal phenomena. While attempts have been made to suppress, experimentally, the central nervous system and the sympathetic chain, nevertheless it has been found impossible to suppress the "localized sensitive complex," which forms an intimate part of the tissue itself.

B. Variations of the sympathetic nerve endings under the action of the estrogens:

In castrated animals the nerve endings are relatively small. Under the action of the estrogens this network increases in size; the interstitial cells grow and develop and true nerve cells appear.

The nerve cells of the peri-uterine sympathetic ganglia in castrated animals are small and retracted. Under the influence of the estrogens Nissl bodies reappear and the nucleus becomes larger and the chromatin more dense. With an increase of the hormonal dose, the cells become two or three times larger than in the normal animal. But the nerve cells grow in size before the

tissues they innervate. The hormone affects first of all the growth of the terminal ganglia, the more so because these ganglia are nearest to the sensitive tissue.

Thus, the destruction of a para-uterine ganglion in the guinea pig produces an asymmetry in the estrogenic reaction: one-half of the vagina will have an estrogenic response, whereas the other half will have no response. The terminal sympathetic cells, i.e., those situated in the wall of the organ itself, form the substratum of these sensitive zones as they react to the hormones, almost completely independently of the remoter sympathetic connections.

C. The chemical intermediary agents:

The nerve endings liberate adrenalin, histamine and acetylcholine. Champy, Samson and Demay were able to obtain utero-vaginal growth phenomena in castrated animals by putting very small quantities of acetylcholine and adrenalin in contact with these organs. But the tissues very rapidly destroy these chemical mediators. To avoid this difficulty it suffices to add ascorbic acid (anti-oxidant) to the adrenalin and eserine (anticholinesterase) to the choline. The effects thus obtained resemble those of follicular hormone.

Coujard has shown that the atrophic para-uterine ganglia in the castrated female remain unchanged after injection of chemical agents, whereas an estrogenic transformation is noted in the vagina.

In the normal animal, the hormones develop first the nerve ganglia and the terminal nervous network. This latter, in turn, throws out into the tissues chemical mediating substances which either potentialize or inhibit the enzymatic arsenal.

The question remaining to be determined is whether there are specialized nerve endings:

adrenalin + acetylcholine = follicular effect,

histamine = progesterone-like effect.

Under luteal action, the same nerve endings, after having secreted adrenalin and acetylcholine, elaborate histamine. At the same time, the Nissl bodies in the nerve cells become rare. Hence, there does not exist a secretory specificity in the nerve endings.

III. CONCLUSION

1. The estrogens have no direct action on the tissues, even though they are necessary to maintain the growth level of explants deriving from embryonic sex organs.

2. The estrogens would seem to act "in vitro" in potentializing, increasing or inhibiting certain enzymes.

3. It is probable that their action "in vivo" is much more complex and is accomplished through the agency of extremely sensitive nerve organs.

Experiments have shown:

(a) that before acting on the receptor tissues, the estrogens act first of all on the trophicity of the cells of the terminal nerve ganglia and

(b) that these latter secrete chemical mediating substances

(c) which then probably intervene in the function of the enzymatic system controlling trophicity, growth and cellular maturation.

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DISCUSSION

LEOPOLD G. KOSS, New York, New York, U.S.A.

This discussor cannot possibly compare his knowledge with that of his most erudite predecessor and could not quote anything either in favor or against the theories proposed and discussed. In spite of the elaborate hypotheses, it would appear that we really do not know anything concerning the mechanism of action of estrogens on the epithelium of the female genital tract. This discussor should merely like to report one observation, namely, that in post-menopausal women an improvement in the degree of maturation of the squamous epithelium is, as a rule, associated with markedly increased vascularity. An outstanding example of this is trichomonas cervicitis, which produces very marked distension of the vessels within the papillae of the squamous epithelium, and is, as a rule, associated with good epithelial maturation and a pseudo-estrogenic effect. If my memory is correct, this was observed before and the writer would appreciate it if the readers would contribute the references which are not available to him.

In summary, it is suggested that at least a part of the estrogenic function could be explained by direct or indirect action of the hormones on the vessels of the epithelium.

CLOSING REMARKS

JEAN DE BRUX, Paris, France:

I am in perfect agreement with my friend, Leopold Koss. Any action able to increase the connective tissue vascularization (steroids, especially estrogens, have this possibility) improve the epidermoid epithelium's trophicity.

However, I wish to add this: the estrogens alone are able to produce a true motivation of the cells, i.e., pyknosis of the nucleus (less than 6μ) and eosinophilia of the cytoplasm.

All other factors (trichomonas, for instance) enhancing angiogenesis give a false maturation by dissociating the motivation of the cell components.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

CYTOLOGICAL CRITERIA OF ESTROGEN EFFECT

MARCEL GAUDEFROY
Lille, France

For the study of estrogen effect on the vaginal epithelium, a constant technique and a definite cytological criteria are necessary. Care should be taken to collect vaginal cells from the inner lateral part of the vagina, the most sensitive zone, and to avoid extraneous causes of error, such as cervical mucus, various infections, latent Trichomonas infestation, vaginal jellies or treatments, etc. On the other hand, a differential stain must be used (Harris-Shorr in our laboratory) and the counting of parabasal and intermediate cells, and the determination of the Karyopyknotic Index (K.I.) and Eosinophilic Index (E.I.) will be affected.

Note: If a nuclear diameter of 5μ is accepted as the criterion of karyopyknosis the E.I. and K.I. are similar, the K.I. being a little higher than the E.I. If a nuclear diameter of 6μ is taken as reference, the K.I. is generally at least 20-30% greater than the E.I.

I consider a nuclear diameter of 5μ to be the best criterion of pyknosis, but a 6μ diameter is more sensitive when used for estrogen estimation. Also, I think that only superficial cells (cells with pyknotic nuclei and eosinophilic cells) are reliable when evaluating estrogen effect.

When studying the proliferative action of an estrogenic drug on the atrophic vaginal epithelium, in which the cells are predominantly parabasal, one may observe the following changes:

(1) The progressive diminution of parabasal and deeper layer intermediate cells, and the production of outer layer intermediate cells (large cyanophilic cells of diameter 30-40μ, with clear vesicular nuclei measuring 9-12μ) and a few non-cornified superficial cells. This transformation will appear much more readily as the posology is higher. This smear type can be maintained by small continuous doses of estrogens.

(2) With stronger doses of various estrogens, we see a highly proliferated cell type, consisting of superficial karyopyknotic cyanophilic cells, and, finally, flat, singly lying, eosinophilic cells, or anuclear squamous cells.

I think that the qualitative colpocytological criterion of estrogen effect is the appearance of the superficial, flat and usually single karyopyknotic cells. It is the best sign of a fine vaginal proliferation.

An approximate quantitative criterion is constituted by the evaluation of the K.I. and E.I., which vary according to the administered quantity of estrogen and the period over which it has been given.

In my routine practice, I do not use the cytohormonal classification of Schmitt nor the cytological evaluation of Wied. I compare the K.I. and the E.I. found during estrogenic treatments with the mean rates of the normal vaginal cycle like Pundel and many other authors, the value of these indices at the time of ovulation being considered as a physiological maximum.

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WOLFGANG KORTE
Bonn, Germany

One knows the chemical formula of 14 to 16 so-called estrogens. However, we know the biological effectiveness of only a part of the above estrogens. Setting aside the gonadotropic hormones which act estrogenic indirectly or which may be transformed to directly effective estrogens, there remain for discussion the "classical" fractions of the follicular hormone: estrone, estradiol and estriol (1).

The estrogenic effect of estradiol is best known. However, only recently it has been found that estriol, which was referred to as an inert metabolite (2, 3, 4, 5) of estradiol, exhibits estrogenic activity of its own (6, 7, 8).

The activity of estrogenic substances must be tested biologically (1). This can be done in human or animal experiments. Most simple and suitable is the Allen-Doisy Test. Since the basic examinations by Papanicoloau (9), the smear technique now provides a very subtle method to observe the degree of maturation of the vaginal epithelium as a reliable indicator of hormonal activity.

The following conditions are necessary for the test: The woman must be in her physiological menopause for several years. The vaginal epithelium must be in the stage of menopausal atrophy. There should not be any sexual intercourse. Uterus and vagina should not be in the stage of prolapse or descensus. Leukoplakias should be absent. Acute and chronic genital infections should be excluded (1).

The cytological criteria of estrogen effect are defined from all methodical, descriptive findings on microscopically examined, fixed and stained vaginal smears. The unfixed specimen examined under the phase microscope is not discussed here. This important technique deserves a separate chapter (10).

The following points are considered on the fixed and stained smear:

1. According to the name the cytological (kytos, logos) criteria refer to description of cytoplasm, nucleus and cellular organelles.

2. Fixation produces (11) comparative structures. Staining makes these structures visible under the bright field microscope. Electron microscopic examination would also deserve a special chapter. One cannot part with fixation and staining in the practical demonstration of the cytological criteria. However, in the strict sense one can consider tinctorial differences only as conditional cytological criteria. Staining permits identification of cytochemical or cytophysical properties of the cells, not structural characteristics.

3. In addition to the epithelial cells, cells from the blood stream and cells from the connective tissue are present. There are also micro-organisms, secretions, minerals and traces of other substances in the smear.

In the following I shall demonstrate the effect of administered estriol on the vaginal epithelium, and I will use the above criteria for the description. I will not discuss the micro-cytometric techniques at this time. In comparative studies it was found that the cellular and nuclear measurements (and the relation thereof) are inferior as compared with the visual optical interpretation by the experienced cytologist (12, 13).

A 58 year old woman (7-para) who was more than 8 years post-menopausal and exhibited an atrophic menopausal type was used for this test. The uterus was in anteflexion, not descended; the adnexa, uterine cervix and vagina did not show any gross pathological findings. There was no sexual intercourse for the last ten years. The woman was admitted to the hospital for this test. Through a period of 14 days we administered daily 1 mg. estriol intramuscularly (Ovestin by Organon). Daily at the same time we prepared a vaginal smear from the posterior wall and the upper part of the vagina. The smears were fixed and stained according to the original technique by Papanicolaou with the modification by Stoll. The dyes used were the ones commercially available from Bayer, Leverkusen, Germany. The photomicrographs were prepared (with the assistance of Miss Waltraut Komp) with the Zeiss-Panphot (periplan-ocular 6x, objective 45x, automatic arc lamp by Gersing). We used 9cm x 12cm plates (Perutz Silbereosin) and printed the photographs on Agfa-Lupex paper.

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* Respectfully dedicated to Professor Dr. W. Ceelen on the occasion of his 75th birthday.

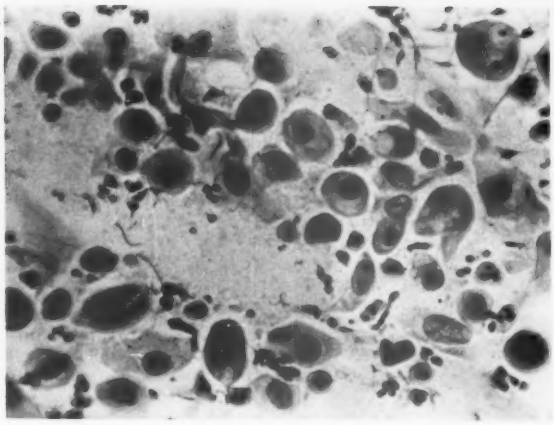

Fig. 1. Control. Slight inflammatory changes, practically a trophic menopausal cell type. In addition to completely atrophic cells, slightly polymorph, slightly hyperplastic (due to inflammatory stimulants) nuclei. Several vacuoles in the cytoplasm. Leukocytes. Short mucous threads. No apparent flora. Staining reaction: pale cyanophilic. (W. Korte)

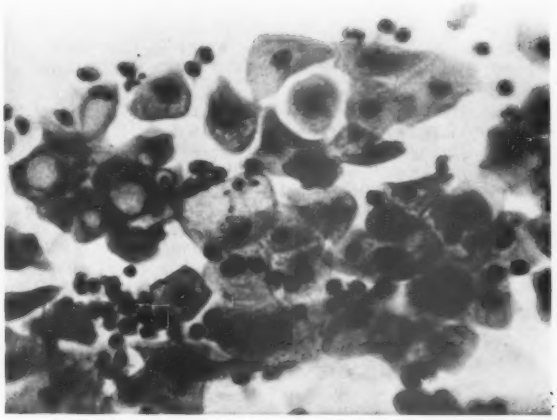

Fig. 2. Two days after onset of medication: after administration of 2 mg. estriol. In addition to single atrophic cells some cells had cytoplasm which contained water vacuoles. First signs of proliferation: cells which compare in their size to intermediate cells. Activation of vascular function: increased amount of leukocytes. Staining reaction: cyanophilic. (W. Korte)

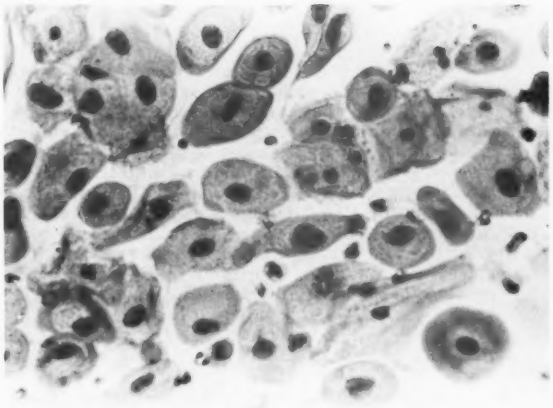

Fig. 3. Five days after onset of medication: after administration of 5 mg. estriol. Still a few atrophic cells present. Predominantly cells which compare in their size to parabasal and intermediate cells, which are really, however, not properly matured, still "half" atrophic superficial cells. Normalization of vascular function: hardly any leukocytes. Staining pale cyanophilic. (W. Korte)

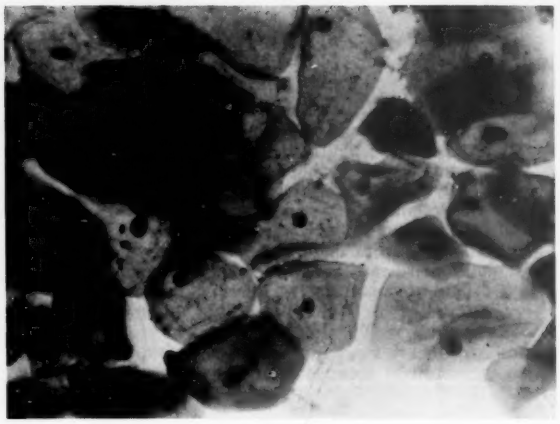

Fig. 4. Seven days after onset of medication: after administration of 7 mg. estriol. The cellular pattern is almost comparable according to cellular exfoliation, structural degree of maturity and tinctorial quality with a (not completely mature) luteal phase. A little more mature than the "mixed type A" by Wied (14). Rare Doederlein's bacilli (short form). No leukocytes. (W. Korte)

Fig. 5. Nine days after onset of medication: after administration of 9 mg. estriol. Only mature, eosinophilic superficial cells (with pyknotic nuclei) which exhibit initial marginal cytoplasmic folding in 30% of the cells. Individual cells lie flat. Leukocytes appear due to local change, see Figure 5a. (W. Korte)

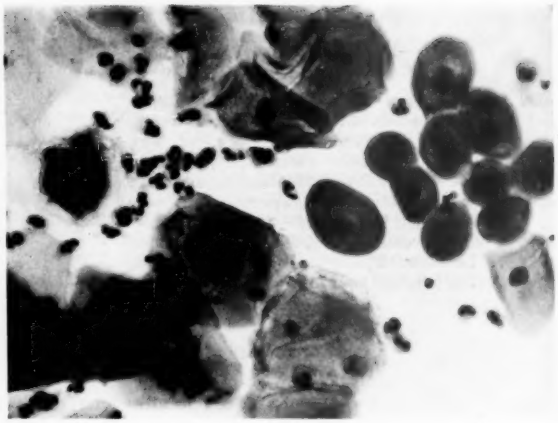

Fig. 5a. Same smear as Figure 5. Some "mature" parabasal cells or just intermediate cells present. The so-called "erosion cells" show that there is also a benign local epithelial lesion with an intercurrent inflammatory reaction present. (W. Korte)

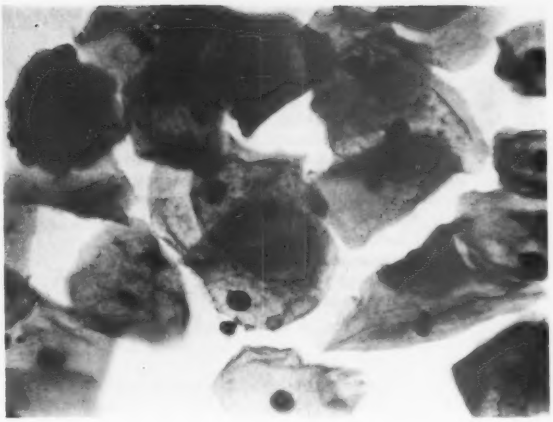

Fig. 6. 12 days after onset of medication: after administration of 12 mg. estriol. One observes a "decrease in maturity" which might be due to the marked swelling effect of estriol. Large, but markedly folded superficial cells, which often lie crowded. Few pyknotic nuclei, relative large nuclei. Predominantly cyanophilic staining reaction. (W. Korte)

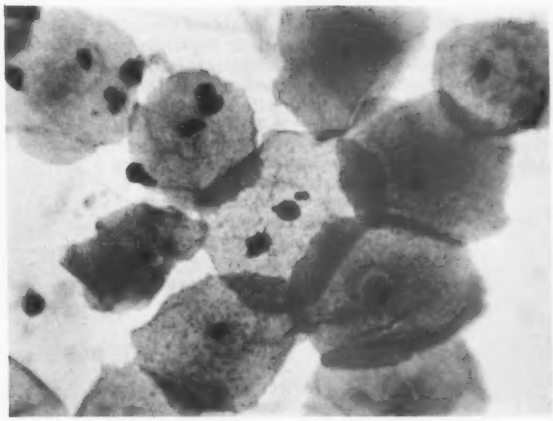

Fig. 7. 14 days after onset of medication: after administration of 14 mg. esteriol. The cellular pattern is compatible with that of a follicular phase during the normal menstrual cycle. The superficial cells are structurally mature, lie flat and are not or hardly folded around the margins, 70% of the cells show eosinophilic staining reaction. (W. Korte)

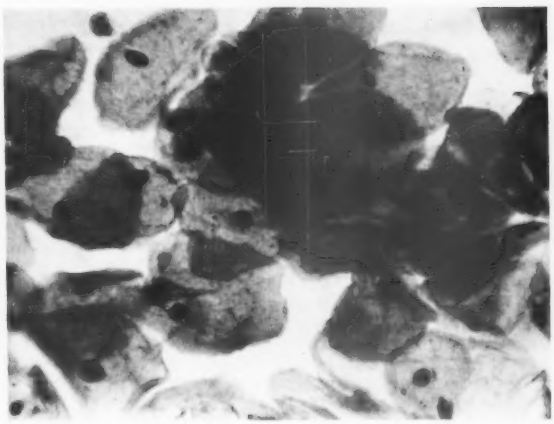

Fig. 8. 16 days after onset of medication: 2 days after termination of medication (total dosage: 14 mg estriol). According to the structure, the tinctorial quality and the disposition of the superficial cells the pattern is compatible with the pattern observed during the early luteal phase after a marked estrogenic effect. A few free nuclei due to cytolysis. Few Doederlein's bacilli, no leukocytes. (W. Korte)

Fig. 9. 19 days after onset of medication: five days after discontinuance of medication. The total amount of cell exfoliation decreases markedly. A few mature superficial cells with little turgor are still present. The cells with pyknotic nuclei become considerably smaller. This corresponds with the first step towards atrophy of the individual cell and the tissue unit. Cyanophilia in approximately 60% of the cells. (W. Korte)

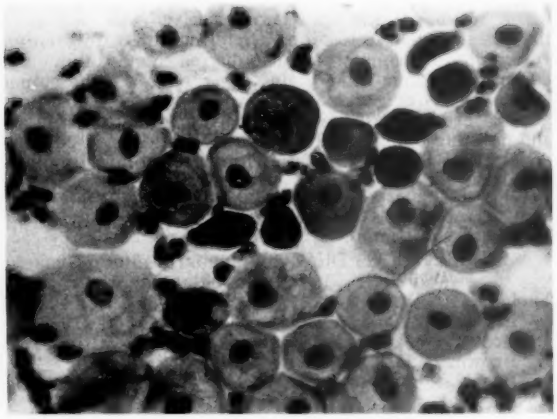

Fig. 10. 26 days after onset of medication: 12 days after discontinuance of medication. The predominant cellular type (90%) according to its cellular size (not its nuclear-cytoplasmic relation) is comparable with the cyanophilic parabasal cells. One really deals here with the less mature superficial cells which became atrophic. (W. Korte)

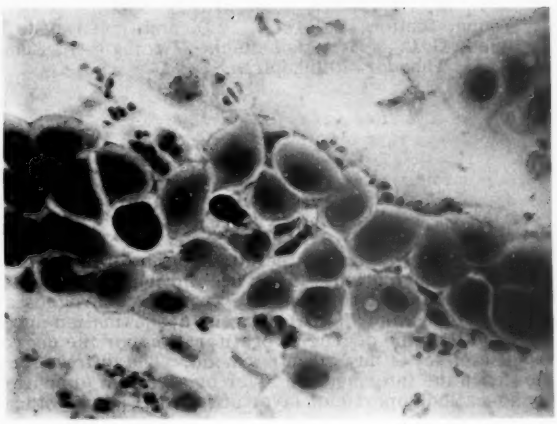

Fig. 11. 45 days after onset of medication: 31 days after discontinuance of medication, very few cells in the entire smear. Single clusters of partially atrophic, slightly inflammatory menopausal cells. "Mucous" secretions, cellular debris, leukocytes in lysis. (W. Korte)

EVALUATION OF FINDINGS AND CONCLUSIONS

1. The effect of estriol on the vaginal epithelium of the postmenopausal woman is demonstrated by means of vaginal cytology. Forty-eight hours after daily parenteral administration of 1 mg estriol each, one finds the first cytological effects. Nine days after onset of medication one can demonstrate a degree of proliferation which is nearly identical with the one during the follicular phase of the normal menstrual cycle.

2. The amount of the administered estrogens seems rather high if one compares estriol with the known threshold dosages of estradiol (at least 350 gamma in nine days). One must consider, however, that estriol is 10 to 20 times biologically less effective than estradiol in regard to the simple estrogenic effect.

3. It is the purpose of the present paper only to demonstrate principally the biological effectiveness of estriol on the vaginal epithelium of the woman. Determinations of the threshold dosages with considerably smaller dosages and comparative examinations with estradiol are being prepared at this time and will be reported in a future paper.

4. It is certainly correct that non-estrogenic hormonal substances may induce stages of proliferation on the vaginal epithelium which may be similar to effects induced with estrogens without the cellular patterns exhibiting the typical estrogenic effect with complete maturation of the epithelium. On the other hand, one must also conclude that there are stages of incomplete maturation of the epithelium during the gradual increase of the estrogenic effect which consist of incompletely mature cellular patterns, and which finally result in a cellular pattern which is compatible with the pattern found during the later follicular phase of the cycle.

5. If one draws comparative conclusions of cytology to histology, then one must conclude that it is advisable to discontinue the separate terminology of basal cells and parabasal cells when referring to atrophic patterns during menopause. There is no intelligent reason (nor is there any comparative condition on any other organ in the human organism) for the assumption that the vaginal epithelium during menopause would successively lose its two upper cellular layers, so to speak like a large erosion. It seems more likely that each layer suffers a loss of cellular amount, cell size, and cellular density. The normally exfoliated cells (without local lesion) correspond to less mature superficial cells, which are completely or incompletely atrophic, i.e., atrophic menopausal superficial cells.

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J. PAUL PUNDEL

Luxembourg, Luxembourg

The vaginal epithelium has the following biological functions: proliferation, differentiation and desquamation. It has been proved that proliferation of the vaginal epithelium can be induced by all the different hormones, estrogens, androgens and progestogens, with direct effect on the end organs (uterus and vagina). As the desquamation accompanies the proliferation of the vaginal epithelium, neither the degree of proliferation nor the degree of desquamation can be considered as a specific effect of any given hormone. The differentiation of the vaginal epithelium starts, as is well known, in the intermediate layer by glycogen formation in the cells and proceeds to the formation of the so-called superficial layer at its final physiological stage. This terminal differentiation is

characterized by the appearance of prekeratin granula in the cytoplasm, paralleling a diminution of the glycogen content, the retraction of the nucleus to complete pyknosis and, finally, the appearance of an eosinophilic reaction of the cytoplasm to differential stains.

Experimental research has shown that this complete differentiation of the vaginal epithelium with karyopyknosis and eosinophilia of the cytoplasm can be obtained in castrated women only with estrogens, while all other hormones like testosterone and progesterone produce only the first step of differentiation with glycogen formation, but are unable to produce a complete differentiation. Some more complex hormones, such as cortisone or androstenediol dipropionate, can induce a minor degree of pyknosis and occasional eosinophilia of the superficial cells, but evidence reveals that these hormones are not exclusively androgens or progestogens, but have poly-hormonal activity.

Unless further experiences prove the contrary, we can, at the present time, conclude the following:

Estrogens are able to induce marked vaginal proliferation and complete physiological differentiation of the vaginal epithelium in the three basic layers with karyopyknosis and eosinophilia of the superficial cells. As proliferation and glycogen formation alone are induced by all other sexual hormones, only the Karyopyknotic and Eosinophilic Indices of the vaginal cells can be considered as specific cytological criteria for estrogen activity.

But the estrogen effect should not be evaluated by using a single criterion, especially not by using only the Karyopyknotic Index. Research done (1, 2, 3) testing progressive dosages of estrogens in the castrated woman has shown that:

1. Pyknosis can be induced without corresponding eosinophilia by low dosages of estrogens. By convenient dosage of estrogens it is possible to induce a Pyknotic Index as high as 90% without any appreciable eosinophilia of these pyknotic superficial cells.

2. Increase of the estrogen dosage rapidly induces an increase of the Eosinophilic Index, and in these cases, the increase of the Eosinophilic Index is accompanied by a lowering of the Pyknotic Index so that the final value of the P.I. remains only slightly higher than that of the E.I.

From these observations one can conclude that pyknosis represents a lower estrogenic level than eosinophilia. If the estrogen stimulation rises above a certain degree, the variations of the Indices are parallel, but if the estrogenic stimulation is low, these Indices can present opposite variations. This means that a high Pyknotic Index is not automatically conclusive for a high estrogenic activity. The latter conclusion is permitted only if the Eosinophilic Index shows correspondingly high values.

Only karyopyknosis and eosinophilia of the superficial vaginal cells can be considered as specific criteria for estrogen effect. The correct evaluation of the estrogen effect can be made only by the simultaneous evaluation of both the Pyknotic and Eosinophilic Indices. The Pyknotic Index alone is not a quantitative index for estrogens, nor is the Eosinophilic Index. The latter has only a diagnostic value if the eosinophilia of the vaginal cells is physiological and not induced artificially by vaginal infections, infestations, or chemical or mechanical irritations. The Eosinophilic Index, on the other hand, can be accepted as a valuable criterion for estrogen effect only if it is based upon the eosinophilia of true superficial cells, i.e., cells containing a pyknotic nucleus as proved by phase contrast microscopy or by exact micrometry (nucleus with a diameter less than 6μ).

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DISCUSSION

JACQUES FERIN, Louvain, Belgium:

We think that the Karyopyknotic Index (percentage of cells whose nuclei's diameters are smaller or equal to 5μ) and the Eosinophilic Index are not too accurate as quantitative indications of estrogenic influence.

A surgically castrated woman has been given various estrogens in increasing dosages during 14 days treatment and we have obtained the following results:

Ethinyl-estradiol:	0.025 mg.:	K.I. 20; E.I. 55
(sublingually)	0.025 mg.:	K.I. 25; E.I. 95
	0.1 mg.:	K.I. 25; E.I. 50

Diethylstilbestrol: (orally)	0.1 mg.:	K.I. 40
	0.2 mg.:	K.I. 40
	0.3 mg.:	K.I. 61
	0.3 mg.:	K.I. 54
	0.5 mg.:	K.I. 64
	0.5 mg.:	K.I. 24
	1 mg.:	K.I. 85
	1 mg.:	K.I. 86
Methyl-bisdehydro-doisynolic acid:	1 mg.:	K.I. 38
	2 mg.:	K.I. 36
	2 mg.:	K.I. 30
	3 mg.:	K.I. 70
	3 mg.:	K.I. 28
	4 mg.:	K.I. 80
	4 mg.:	K.I. 85

EMMERICH VON HAAM, Columbus, Ohio, U.S.A.:

I agree with Gaudefroy that a careful standardized technique is one of the most important requirements for a reliable estimation of the estrogen status of a woman by cytological criteria. We prefer the differential count of a hundred single and well-stained cells and express the status of estrus in a woman by 0 to 3 plus. We feel that this method can be taught easier to cytotechnologists than the micro measurements necessary for determination of the Karyopyknotic Index. I agree with Korte that an experienced cytotechnologist can do better by relying on the cell picture as a whole rather than using indices and measurements. Pundel's emphasis that Karyopyknotic and Eosinophilic Indices are the only specific cytologic criteria for estrogen activity and that both must be used together in order to make a correct evaluation of the estrogen status is interesting. We have occasionally seen a highly estrogenic smear in a woman 70 years and older who has been in menopause for sometime and has definitely not received any estrogenic therapy. This we explained as effect from estrogenic adreno-corticoids and presently are correlating those findings with other clinical symptoms of hyperadrenocorticism.

GUILLERMO TERZANO, Buenos Aires, Argentina:

Provided that smears have been correctly taken and properly stained, the Karyopyknotic Index and the Eosinophilic Index are the most reliable qualitative criteria of estrogenic effect. The relationship between both indices will vary according to the estrogenic (endogenous or exogenous) effect upon the vaginal epithelium (marked, moderate, or slight).

As for karyopyknosis as a specific criterion for estrogenic effect, it is my opinion that the Karyopyknotic Index may be influenced by hormones other than estrogens.

ERICA WACHTEL, London, England, U.K.:

It is difficult to add to the excellent and precise contributions of Pundel and Gaudefroy. I fully agree with their findings, but both authors have been dealing with ideal cases, i.e., the effects of estrogens administered to healthy, postmenopausal women. If we consider the cytological criteria of estrogen effect in the gynecological patient, the value of the Eosinophilic Index becomes doubtful. In my opinion the degree of eosinophilia in a film is only partially related to its estrogenic inference and depends to at least an equal degree on the pH of the vagina. Infections, chemical agents used in douches, cohabitation, etc. will, therefore, materially influence the staining properties of the cells and, if we accept the Eosinophilic Index as an important guide in our assessment of estrogenic activity, we will probably often be misled by it. I agree that for research purposes and for the testing of estrogenic preparations the Karyopyknotic as well as the Eosinophilic Index should be determined in the manner described by Pundel, using for the tests healthy postmenopausal women not practizing coitus. However, for the purpose of estimating estrogen effects in the gynecological patient in whose hormonal pattern we are interested I would suggest relying solely on the prevalent cell type in the smear and the Karyopyknotic Index.

CLOSING REMARKS

MARCEL GAUDEFROY, Lille, Nord, France:

I wish to thank the authors who have kindly added some refinements to my paper. Von Haam emphasizes the difficulties in measuring the Karyopyknotic Index. We admit that it is not useful for karyopyknotic cell counts, some experience provided, to make measurements on every single nucleus. The point-like aspect of the nucleus (stained with Harris hematoxylin followed by Shorr's stain), added to the complete disappearance of any nuclear structures, is sufficient criterion of karyopyknosis. Counts made according to these criteria, compared with ones made by cytometric measurements, differ only by 3-4%. Therefore, the method is accurate enough for clinical purposes.

I believe, as does Erica Wachtel, that the count of eosinophilic cells could cause some error and I agree with Terzano who insists upon the necessity of correct collecting and staining of the smears.

Finally, Ferin reports counts which he interprets as in contradiction to the application of the quantitative vaginal cytology for the diagnosis of the amount of estrogenic substances. If one excludes the treatment with ethinyl-estradiol which did not last more than 3 days, one should observe that under treatment with diethylstilbestrol or methyl-bisdehydro-doisynolic acid the Karyopyknotic Index progressively increases with the increase of the dosage and the duration of the treatment. Some "lows" in the Karyopyknotic Index appear during the above treatment and cannot clearly be explained; these drops do not at all influence the steady increase of the Index. A possible explanation could be assumed in some metabolic breakdown processes (in the liver) which lead to decomposition of the administered estrogens and result in considerable decrease of the blood level of circulating estrogens.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

From the main papers and from the discussions we can accept the conclusion that the Eosinophilic and the Karyopyknotic Indices are the most valuable qualitative and quantitative criteria for estrogenic effects. To Wachtel I must reply that my findings are not based exclusively on ideal cases. My conclusions are gathered from routine gynecological material and I was not able to find important differences between the reactions in postmenopausal normal women and patients with various menstrual disorders. However, I agree completely with Wachtel that the routine patients must be carefully selected for experimental work because of the numerous possible factors which can produce some modification of the Eosinophilic and even of the Karyopyknotic Index. One cannot repeat sufficiently to the beginner all of these possibilities of error which should be carefully considered before one makes any hormonal diagnosis. The most frequent changes are seen in the Eosinophilic Index, for the reasons indicated by Wachtel. Other factors which can interfere with the Karyopyknotic Index are presented elsewhere in this symposium. Furthermore, one should not forget that the hormonal physiology of women cannot be described strictly mathematically, and that, likewise, the vaginal hormonal diagnosis cannot be based on purely numerical criteria. I think that the experience of a cytologist is proven if he knows: (1) in which cases he can safely use numerical criteria such as the Eosinophilic and Karyopyknotic Indices with diagnostic accuracy, (2) in which cases he has to evaluate the smear by other criteria and (3) when he has to apply some correction factors in order to avoid errors due to non-hormonal modifications of the vaginal cytology.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

HISTOLOGICAL CRITERIA OF ESTROGENIC EFFECT

JEAN de BRUX
Paris, France

The physiological and experimental effects of estrogens are, at present, fairly well known, and their practical application has gained recognition in the routine use of the endometrial biopsy. We shall discuss here only the action of the estrogens at the vaginal and ectocervical levels.

In prepubescence, as in the menopause, there exists only a single layer of flattened cells with large nuclei, uniformly chromatic, with relatively little cytoplasm, and without mitosis. In the menopause, these cells are extremely fragile and feebly retained by the intercellular digitations of the basal membrane, their exfoliation exposing a chorion which generally becomes infiltrated, quite locally, by elements of subacute inflammation.

The ingestion of estrogen in the menopause, or the first follicular secretion in puberty, produces:

(a) a cellular activity, manifested by nuclear hyperplasia, with increasing charge of DNA (Feulgen +); and

(b) mitotic figures, whose multiplicity and rapidity of appearance increase proportionately with a rise in the estrogen level—with allowance for (1) individual variations and (2) maximum-minimum threshold limits of the hormone.

The cellular stratification arising from this activity generally establishes itself in an orderly fashion, with progressive differentiation of intermediate cells, the cytoplasm becoming flattened, whereas the nucleus retracts gradually, becoming pyknotic; these cells are later exfoliated in the form of acidophil CSNP. Or, at a more advanced stage, the nucleus disappears and free keratinized cells are exfoliated; very large doses of estrogen are necessary to obtain this result. Nevertheless, it should be noted that an excess of estrogen never produces a leukoplakia. This latter is the product of an organic lesional state, and not that of an excess of hormone.

Estrogenic action, therefore, may be summarized as:

(a) a growth determinant acting on the vaginal epithelium, and

(b) a determinant of differentiation acting on the elements thus produced.

Study of this differentiation

In the basal layers, the nucleic acid level—in particular, that of RNA—is very elevated. At the malpighian levels, we find a very notable change of amount of saccharidic substances (mucin and glycogen). It may be wondered why these substances accumulate in this layer. Certain biochemists believe that the deposit of these carbohydrate substances is a phenomenon resulting from the decrease in O_2. Dempsey and Wisloki have demonstrated an inverse relationship between the deposit of glycogen and the activity of aerobic oxidation. In this connection, Davis, Meyer and McShan have shown that the estrogens inhibit the cellular oxidizing enzymes, which may explain the accumulation of polysaccharidic and saccharidic substances at the intermediate cell level. These facts are perhaps not exclusively the results of the estrogens: the other steroid hormones —progesterone and androgen—also have the property of producing the proliferation of the vaginal epithelium. But the most significant characteristics of the estrogens are:

(a) the property of inducing nuclear pyknosis, and

(b) the production of kerato-hyalin granules, resulting in total maturation of the squamous cells.

In fact, while the other steroid hormones are likewise capable of determining the growth of the vaginal epithelium, nevertheless, only the estrogens produce complete cellular maturation, manifested first by the pyknosis and later by the disappearance of the nucleus, the cytoplasm containing kerato-hyalin granules which modify its chromatophilia by the formation of sulfhydryl and disulfide radicals.

In certain organic lesions, a deviation in the utilization of nutritive elements, caused by disturbance of enzyme action, terminates in the non-formation of glycogen, with appearance of grains of eleidin in the upper portion of the epithelium. However, here the estrogen no longer acts directly or, in any case, its action is interfered with by other factors.

Action of the estrogens on the connective tissue of the cervix and the vagina

The connective tissue of the cervix (the most readily studied because of the large number of cervical biopsies performed) consists of:

- the basal connective tissue and
- the basal membrane.

The connective tissue is composed of argentaffin and collagen fibers in a medium of ground substance of variable density. In the deeper portions the two varieties of fibers are rather loose and the ground substance is dense. In the sub-epithelial region the fibers present a thicker and more packed aspect.

In the cervix of the menopause, the ground substance, which predominates, appears sclerotic, the collagen fibers are retracted and small, and the reticulin is thin and scanty, localized in the sub-epithelial area, forming grayish zones elsewhere. The PAS reaction, as modified by Hale and by Retter and Oleson, is feeble, and shows that this ground substance is composed of very polymerized polysaccharide acids; incubation with hyaluronidase has practically no modifying effect on the intensity of the PAS. The vessels are rare, their walls thickened, and their lumen markedly stenosed. The basal membrane is regular and thin, with very short or no intercellular digitations. The rare cells encountered are small fibroblasts and very occasional mast cells.

With the injection of estrogens, this aspect will be considerably modified:

(a) The ground substance becomes edematous, and the PAS reaction turns very strongly positive, testifying to the depolymerization of the ground substance.

(b) The collagen fibers widen and take on a florid aspect.

(c) The reticulin reappears distinct, slender, regular and branched.

(d) The fibrocytes become fibroblastic, with PAS-positive granules in their cytoplasm. Finally, especially in the sub-epithelial zone, mast cells appear.

(e) The basal membrane widens, but its boundaries become indistinct and its reaction to the PAS is less markedly positive. This aspect of the basal membrane is the proof of the condensation of glycoproteins, serving as a zone of exchange between the connective tissue and the epithelial layer.

(f) Finally, there appear numerous newly-formed capillaries which "rise" toward the surface and, curving, follow along the basal membrane.

These, then, are the manifestations of estrogenic action on the connective tissue:

(a) depolymerization of the ground substance, with rejuvenation of the collagen fibers and formation of argentaffin fibers;

(b) cellular proliferation and formation of granules, testifying to their functional activity (mast cells).

(c) reappearance of angiogenesis.

We should note here that this action of the estrogens applies only for a certain dosage of the hormone. Given in very large doses, the results are different:

(a) The depolymerization becomes such that the connective tissue becomes edematous, with dispersion therein of the collagen fibers. The PAS becomes very faintly positive, sometimes even negative, and the reticulin disappears or becomes thin and very scanty.

(b) The cellular proliferation and the angiogenesis stop, giving an aspect resembling that obtained by the administration of cortisone.

Conclusion:

Is there a specific action of estrogen upon the cervico-vaginal epithelium and connective tissue ?

(a) On the epithelium: Only the nuclear pyknosis and the acidophilia of the cytoplasm are the properties peculiar to the estrogens.

(b) On the connective tissue: All the steroid hormones induce almost the same trophic effects on the connective tissue, but the homeostasis is probably different for the various hormones. Our knowledge in this matter is still precarious and insufficient.

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HERBERT LAX

West-Berlin, Germany

Among numerous other effects, estrogen administration produces a general increase in metabolism and in cellular proliferation. This effect is especially evident in the organs of the female genital tract. Other substances, such as the stilbenes and those which are not phenanthrene derivatives may induce these effects. Each tissue in the genital tract reacts in its specific and characteristic manner: e.g., the tubal epithelium reacts differently from the endometrium and the latter, again, reacts differently from the vaginal epithelium. Thus for the realization of the "estrogen effect," the functions of the endocrine receptors will play a decisive role. Even in steadily increased administration of estrogens one finds that the increase in proliferative effect is limited. Why this occurs is not known since we know practically nothing about the exact mechanism of the estrogen activity, a considerable deficiency in our knowledge.

The estrogen effect is histologically recognizable on the general criteria of tissue growth.

The musculature of the tube and uterus grows, i.e., the nuclei and cytoplasm of the muscle cells increase in size; increase in estrogen dosage is accompanied by a limited increase of the muscle cells through mitotic division. In the tube one finds stratification of the epithelium with increase of the ciliated epithelial cells. The non-ciliated epithelial cells undergo only slight development. In the endometrium the "functionalis" is particularly affected, i.e., the superficial zone which reacts to administered estrogens, whereas the "basalis" which lies next to the musculature exhibits only insignificant estrogen effect. This is again a sign for the significance of the special cellular function of the receptors for the realization of the estrogen effect. The epithelial cells of the endometrial glands become larger, highly cylindric, and are increased by mitotic division—depending upon the amount of estrogen administered. The cells of the monocellular epithelial margins seem to be pressed together. Where the compression seems to be slight, one finds an increase in nuclear volume and nuclear clarification. One finds histochemically abundant DNA and RNA alkaline phosphatase in the cytoplasm of the proliferating epithelial cells as a result of the increased protein synthesis. The glandular lumina appear stretched. The stroma grows also. The stromal nuclei become larger and looser. The increase of the argyrophilic fibrils is characteristic, and also dependent on the administration of estrogens. The development of the vessels is synchronized with the growth of the glands and the stroma. The vessels run in a slightly sinuous line to the surface; formation of new capillaries can be found. The individually variable increase in vascular permeability is responsible for fluid extravasation into the stroma, which can be increased to edema, as is found also in the subcutaneous tissue. This increased fluid content together with the true growth is responsible for the height of proliferation of the endometrium under estrogen influence. Long-lasting estrogen medication results in an overly increased endometrial growth. The mitoses in the epithelial cells increase. The glands are often more involved than the stroma, which results in an irregular process with cystic dilatation of the glandular lumina: glandular hyperplasia. Each degree of proliferation needs an adequate amount of estrogen for its development and maintenance. The mucous membrane breaks down despite persistent estrogen administration if the maximal degree of proliferation is reached.

The estrogen effect on the vaginal epithelium is recognizable by the stratification and maturation of the squamous epithelium. Even in cases of extremely marked atrophy one finds epithelial growth from the stratum germinativum to the normal height after estrogen administration.

Except for the basal and parabasal cellular layers, the epithelial cells of the vagina produce very much glycogen, approximately ten times more than those of the epidermis.

One can determine "estrogen effect," therefore, only by the height of proliferation. For the histologist there is no possibility to identify a slight proliferation as "androgenic effect" or "progestational effect." Cytology permits finer differentiation of the cellular criteria. It is, however, only possible to distinguish these cellular criteria if the cells are liberated from the fixed tension within the tissue, and—when through this—the true cellular forms and features become apparent.

DISCUSSION

JEAN BERGER, Basel, Switzerland:

One can study estrogenic effects best on squamous epithelium of the vagina or cervix of postmenopausal women. Here I agree with Lax that exfoliative cytology gives criteria of the hormonal influences. The estrogen effect in histology can only be recognized by the proliferation of the epithelium. Histochemical reactions and colorations give much better criteria of those effects, as de Brux and others showed. In examining histochemical reactions of the squamous epithelium of the vagina and portio in 30 healthy postmenopausal women (aged between 49 and 68 years, postmenopausal for from two to 18 years) we found two groups:

(a) postmenopausal women without estrogen effect (smears showed an absolute atrophy with small, round, basophilic basal cells).

(b) postmenopausal women with a low estrogen effect (eosinophilic and pyknotic effect up to 25%).

In comparing the histochemical reactions in these two groups, we found, in the second group, a proliferation of the epithelium and a well-marked increasing acid phosphatase (Pearse) corresponding to the estrogen effect. Acid phosphatase activity could be seen, above all, in the cytoplasm and nuclei of the basal and parabasal layer. The higher the estrogen effect is, the better RNA and DNA can be differentiated in the lower layers (zone of growth). The neutral mucopolysaccharides (PAS-reaction by McManus/Hotchkiss) are well localized and differentiated when an estrogen effect exists. In the atrophic type this last reaction shows only a diffuse localization and no differentiation.

The comparative examinations show the importance of histochemical reactions to recognize the estrogen effect on squamous epithelium. These reactions complete and agree qualitatively and quantitatively with the results of exfoliative cytology.

WOLFGANG KORTE, Bonn, Germany:

To Dr. de Brux:

Histological criteria of the estrogen effect relate to the formal genesis, to the structural condition and to the morphologically demonstrable ability of function of a tissue formation. The tissue can be defined as a formation of cells which are differentiated in the same way. By differentiation I understand:

1. the development by growth of the single cell and of the cell formation especially tied to the nucleus.

2. the formation of the real ability of function of the cell and of the tissue especially tied to the protoplasm.

3. the real function-work of the cell (of the tissue) to exhaustion.

Estrogens start such processes of differentiation. Here we should not discuss whether they do it unaided or whether they gear into processes of maturation which are stimulated in another way. An atrophic vaginal epithelium still exists as tissue without estrogen stimulus. If estrogens are administered, the tissue starts growing. This presupposes that a potency of growth tied to the cell or to the tissue still exists in the atrophic epithelium. As we know of all tissue and of other organs, growth takes place according to fixed laws which the organism in non-pathological conditions can only follow in the same way (even though in a certain scope of variation).

Estrogens are certainly able to effect the growth of the cell and of the tissue (growth determinant of de Brux). One can ask: Do estrogens stimulate only the real process of growth of cells and tissue? Are they also necessary for the function-work after the end of the period of growth? Do they influence the start and the course of the bionecrosis, of the resorption or of the desquamation of the cells after the function-work is finished? Probably the stimulation up to a certain degree of differentiation is enough to automatically bring about the continuation of the further processes of life of the cells in a suitable medium. Consequently, one cannot term every

moment and every form of structure of the development of the cell or of the tissue as directly estrogen-effected. This can be shown on atrophic epithelial cells which no longer have any potency of growth and show changes of plasm upon estrogen medication, which must be nearly interpreted as pathological ones (hydropic swelling without any function-work). It would be very interesting to interrupt the supply of estrogens in a tissue culture at that very moment when the nucleus-tied tendency of proliferation has reached its culmination. The secretion—or on the vaginal epithelium, the so-called holocrine function of glands—is, in a suitable culture medium, the automatic consequence of the end of growth. Besides the known reactions, in the main, cytologically demonstrated processes of maturation, one can add for the tissue-picture of the vaginal membrane the formation of the papillary body in a typical manner by estrogen activity. Enlargement of the epithelial mass and of the surface results in the formation of folds of the vaginal membrane.

What is correct for the surface coat of the pavement epithelium one can allegedly compare with the cervical glandular tissue. The proliferation operates not only on the single cell. New glands originate, for the present formed as solid epithelial buds, and gear into the process of secretion after the end of their phase of growth.

The connective tissue of the vessels reacts differently. The hyalinized fibers swell (water), the frequently fragmented elastic elements too. But just as new connective tissue cells, fibroblasts, fibrocytes and histiocytes appear, new fibers are also formed. The hyperemia of the vessels results not only in serous, but also in cellular excretions. So-called inflammation cells from the blood vessels emigrate or are pressed through the loosened cement-substance lines of the vessel walls and can be shown in the mesenchyme, which is abounding in water. This happens, if one thinks teleologically, as long as a medium has been reached which gives to the glandular cells (which have been proliferated in the meantime and are ready for secretion) the possibility of taking up enough substances for their effect of secretion.

To Dr. Lax:

To evaluate estrogen effects histologically, it is necessary to consider some factors:

1. the quantity and kind of the administered substance,
2. the space of time in which it can become effective,
3. the state of the tissue before medication,
4. the conditions of reaction, regeneration and growth of different tissues.

The degree of differentiation of different tissues and of their subspecies in organs requires different threshold limits to be able to show an estrogen effect. Highly differentiated tissues, with relatively slow growth and quick death of the tissue and the cells, need quantities of substance and periods of action other than more primitive, quicker growing tissue formations. Thereby, the different formal and qualitative reaction, e.g., of the various sorts of epithelium in the genital region, is explained. In which morphological manner the tissues become formed may be dependent on the kind of estrogens. Different estrogens are able to influence single tissues of an organ (e.g., myometrium, endometrium, cervical epithelium of the uterus) differently. Estradiol in a fixed dose, for instance, brings the endometrium and, earlier, the more primitive vaginal epithelium to the height of proliferation. Estriol causes a maturation of the vaginal membrane, but one cannot speak of an approximately comparable effect on the mucous coat of the uterus. The stimulus of proliferation is common; time factor and threshold limit are different. I think that the topography of the tissues in question is not decisive for this, but rather, their potency of growth and reaction as fixed genetically.

I am not able to consent to the interpretation of Lax that the basalis of the endometrium exhibits only insignificant consequences of an estrogen effect. The basalis is the carrier of the potency of growth of the mucous coat of the uterus. Consequently, the essential histological criteria of the estrogen effect must be found there. This can be shown in the atrophic menopause by small doses of estradiol or by administration of large quantities of estriol. Before a functionalis becomes visible with such a biologic test, one sees a new formation and strong proliferation of the basalis glands. Similarly, the "non-participation in the estrogen effect" of the tubal epithelium without kinocilia may be explained. Here, however, are not progressive processes but regressive ones. Such cylindric epithelial cells may have already exceeded their culmination of proliferation. The loss of kinocilia may be already a physiological degree of degeneration. The potency of reaction of these cells to respond visibly to an estrogenic stimulus is no longer as great or is entirely gone. This cell formation is consequently hardly suitable to show an estrogen effect.

The last word about long-lasting estrogen medication and its effect on the endometrium has perhaps not yet been spoken. Cyst formation and tendency toward necrosis of the glandular-cystic hyperplasia of the mucous membrane are ensuing conditions of an over-increased and disturbed proliferation which is no longer subordinate to the estrogen effect. One can produce such

results by doses of estriol, which causes normal proliferation of other tissues. Type, composition and biologic quality of estrogens consequently may play a role in the pathological findings.

The estrogen effect on the vulva is histologically demonstrable partly in the vaginal epithelium, partly at the pavement epithelium of the skin. The quantity of the needed effective substances for women in the menopause is disproportionately high on the vulva in comparison with the other genital regions. This is perhaps the consequence of incomparably bad vascularization. Perhaps relatively strong regressive sub-epithelial changes play a role. They can, as we know from experience, only be influenced by estrogens during longer periods of medical care.

CLOSING REMARKS

JEAN DE BRUX, Paris, France: no comments

HERBERT LAX, West-Berlin, Germany: no comments

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THE POLYSACCHARIDE CONTENT OF THE HUMAN VAGINA AS A CRITERION OF ACTION OF SEX HORMONES

JOSÉ BOTELLA LLUSIA, F. NOGALES and LUIS MONTALVO RUIZ
Madrid, Spain

Past, well known investigations (4, 7, 12) have demonstrated the presence of glycogen in the human vaginal epithelium and have related it to estrogenic influence. Consequently, methods have been tried to measure this estrogenic activity, ways capable of detecting this polysaccharide in the desquamated cells (6, 9, 12). Nevertheless, some contradictions observed later (9) have instilled doubts on the relationship between estrogens and the glycogenic test in vaginal cytology.

In animals, glycogen is not the only polysaccharide which is found in the vaginal epithelium, a mucification (mucopolysaccharide) having been described in the superficial layers of the female mouse (11), rat (3) and guinea-pig (8). The histochemical studies on these animals suggest that glycogen storage is related to estrogenic action, while the mucification is due to progesterone.

In previous investigations (1), we have been able to demonstrate the existence of a superficial mucification in the human vagina. The samples stained by the Periodic-Acid-Schiff method (PAS) reveal a PAS positive substance, amylase resistant, on the superficial corneus stratum (10) while in the intermediate zone there are, especially in young women, great quantities of a PAS positive substance, amylase-soluble (glycogen) (13). We have described thus, just as in the rodent, a "superficial mucification of the human vagina."

The cyclic changes in the mucopolysaccharide and glycogen content have been studied by Nogales and Botella (1). The results can be seen on Table 1, where it is demonstrated that the glycogen predominates during the first half of the cycle, while in the second half there is a predominance of the mucin.

The histochemical changes in the vaginal smear during the cycle have been studied by Montalvo (2). The results are shown in Table II. The author (2) indicates that the glycogen is not found in the most superficial layer, but, on the contrary, is found in the intermediate zones of the vaginal epithelium. Hence, only during the period of the cycle in which the intermediate cells appear in great quantities is the amount of vaginal glycogen high. The administration of estrogens induces an increase in the exfoliation of aglycogenic superficial cells and, hence, is not followed by an increase in the amount of glycogen of the vaginal desquamation (although glycogen is found in the epithelial walls, as shown in Table III).

Cytochemical studies relating to mucin and glycogen storage in surgically castrated women have been made by Dominquez-Adame (5) in our group. The results summarized in Table III show the following:

1) Estrogens increase: a) the thickness of the vaginal wall. b) the thickness of the glycogenic layer. c) the intracellular content of glycogen.

2) Progesterone increases: the mucopolysaccharide content of the superficial (karyopyknotic) layer. It does not increase the glycogenic layer.

3) Androgens behave just like progesterone, but with a weaker action.

The estrogens increase the storage of vaginal glycogen, but only in the stratum intermedium. For this reason, the estrogenic action is not manifested by an increase in the glycogen content of the desquamated cells. Consequently, the glycogenic index is not demonstrative of

estrogenic action. The glycogen is a histochemical, rather than a cytological, demonstration of estrogenic action on the vaginal epithelium.

Progesterone increases the mucin content of the superficial cells, giving rise to superficial mucification. This action, which is well known in rodents, also takes place in women. Since this action influences the superficial cells closer to desquamation, it is possible that this mucification causes the cells, under the action of progesterone, to appear folded on the smear (due to the action of the fixative on the mucin) and crowded (increase of the mucous interstitial substance). It is possible that the gestagenic reaction of the vagina is the simple cytological result of the superficial mucification phenomenon.

TABLE I

PAS REACTION ON THE DIFFERENT DAYS OF THE CYCLE OF NORMALLY OVULATING WOMEN

Day of the cycle	PAS without digestion				PAS with amylase digestion			
	Negative	+	++	+++	Negative	+	++	+++
	Number of cases							
1 - 5 (10 cases)	0	1	6	3	7	3	0	0
6 - 10 (15 cases)	2	3	5	5	11	2	1	1
11 - 15 (14 cases)	1	4	4	5	9	2	2	1
16 - 20 (15 cases)	0	4	4	7	1	5	4	5
21 - 25 (20 cases)	2	6	5	9	3	6	5	6
26 - 28 (15 cases)	0	4	5	6	1	3	6	5

TABLE II

PAS REACTION IN VAGINAL SMEARS IN RELATION TO THE PAPANICOLAOU TEST

Type of smear	PAS without digestion				PAS with amylase digestion			
	Negative	+	++	+++	Negative	+	++	+++
	Number of cases							
Days 1-15 Hypofollicular (10 cases)	0	3	3	4	9	1	0	0
Follicular (7 cases)	5	1	1	0	7	0	0	0
Hyperfollicular (5 cases)	4	1	0	0	4	0	0	0
Days 16-28 or more Luteal (10 cases)	0	3	5	2	3	0	5	2
Hypoluteal (6 cases)	0	0	3	3	6	0	0	0
Follicular-luteal (4 cases)	0	0	0	4	0	0	1	3

TABLE III

PAS REACTION IN THE VAGINAL MUCOSA OF CASTRATED WOMEN
30-60 DAYS AFTER CASTRATION

Hormone administered	PAS without digestion				PAS with amylase digestion			
	Negative	+	++	+++	Negative	+	++	+++
	Number of cases							
None (20 cases)	5	6	9	0	4	7	8	1
Estrogen Estradiol 5 mg. (10 cases)	0	5	1	4	10	0	0	0
Estradiol 10 mg. (10 cases)	0	2	2	6	10	0	0	0
Progesterone 50 mg. (10 cases)	4	2	3	1	3	2	3	2
100 mg. (10 cases)	3	1	5	1	3	1	5	1
Testosterone 50 mg. (5 cases)	0	1	2	1	1	1	2	1
100 mg. (5 cases)	0	1	3	1	1	1	2	1

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DISCUSSION

ALVAN G. FORAKER, Jacksonville, Florida, U.S.A.:

Undoubtedly the relation of glycogen and other polysaccharides to vaginal and cervical squamous mucosa, as regards estrogenic influence, and distribution in normal, atypical and neoplastic growth is much more complex than has been supposed. Glycogen is not a simple and wholly reliable index of maturation, of estrogen effect, or the absence of neoplastic growth. Four studies from our laboratory may cast some additional light on the problem. As anticipated a greater percentage of glycogen containing cells was found in vaginal smears of pregnant women than of non-pregnant women (1). This follows orthodox views on the subject. Re-examination of the data of that study in the light of Botella-Llusia and co-worker's comments on superficial mucification of the human vagina does not show any material differences between the pregnant and non-pregnant women as regards percentage of PAS positive, ptyalase resistant material. We failed to find (3) evidence of differences in glycogen or "mucopolysaccharide" staining of urine sediment epithelial cells in groups of pregnant and non-pregnant women. Stilbestrol (2) appeared to reduce the percentage of glycogen containing cells in vaginal smears of pregnant women, in comparison with untreated pregnant women. We have doubts (4) as to the validity of glycogen presence or absence as a reliable means of distinguishing between non-neoplastic and neoplastic proliferating cervical

squamous mucosal cells. The postulates and studies of Botella-Llusia and co-workers are of much interest. Further work in this area should clarify many of these unsettled points.

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EMMERICH VON HAAM, Columbus, Ohio, U.S.A.:

The attempted differentiation between the estrogen effect and the progesterone effect on the vaginal mucosa by utilizing the two variations of the PAS stain for mucin and glycogen seems quite simple and is convincingly demonstrated in Table I. Table II shows a good contrast between the follicular and hyper-follicular cases (first part of the cycle) and the lutein cases with regard to mucin production. The effect of administering various hormones can also be studied by this method (Table III). This method should be valuable in sterility tests and in examination of habitual abortion.

CLOSING REMARKS

J. BOTELLA LLUSIA, F. NOGALES and L. MONTALVO, Madrid, Spain:

Dr. Foraker states that the glycogen content of vaginal and cervical smears in pregnant and non-pregnant women is apparently not proportional to the level of estrogenic activity. The same observation was made by Montalvo (1) many years ago. The observations of this author have shown that in new-born girls, the increase in the amount of vaginal glycogen, as measured in the desquamated cells, was in relation to an estrogen deprivation and not to an estrogen increase. In many cases, treatment with stilbestrol and with other natural or artifical estrogens decrease the glycogen content of the desquamated cells. If, on the other hand, we study the glycogen content of the vaginal wall in histologic preparations, we can observe that regularly estrogen increases the glycogen content. Since the histochemical methods reveal the highest content of this polysaccharide not in the superficial layer but, on the contrary, in the intermediate one, it is easy to understand why estrogens do not increase the glycogen content of the smear. This hormone group produces a storage of glycogen in intermediate layers, but increases only the desquamation of the superficial cornified cells which usually do not contain any or a very scarce amount of this substance. Undoubtedly, much further work is necessary to resolve these problems.

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CYTOPLASMIC GRANULES AND ESTROGEN EFFECT

HERBERT E. NIEBURGS and HOWARD S. ZUCKER
New York, New York, U.S.A.

The significance of cytoplasmic granules in vaginal smears is not well understood. Cytoplasmic inclusions may be of endogenous or exogenous origin. Endogenous cytoplasmic constituents arise from the metabolism of the cell, while exogenous inclusions occur through phagocytosis or pinocytosis. Cytoplasmic pigments may represent lipoid, carbohydrate, hematoidin, melanin and others.

The cytoplasmic inclusions found in vaginal smears are granules or globules. The cytoplasmic granules usually stain the same color as the pyknotic nuclei of superficial cells. They appear dark brown or black when stained by the Papanicolaou method. The size of the granules is usually smaller than that of the nucleus with moderate variation in size. At times, one of the granules may be adjacent to a pyknotic nucleus of about the same size. Cytoplasmic globules are usually larger than the granules and stain lighter than the nucleus. Their size is either uniform or varies slightly.

The cytoplasmic granules are the ones more frequently seen in eosinophilic superficial cells with pyknotic nuclei of vaginal smears. Since they occur in these cells, their presence is apparently in close relation to estrogenic activity. Thus, they may be found during the latter part of the proliferative phase, or during prolonged estrogen administration or continued intrinsic estrogenic stimulation.

The presence of cytoplasmic granules has been described by Ayre (1) as one of the criteria for the diagnosis of the so-called "precancer cell complex" (nearo-carcinoma). An increased number of cells with cytoplasmic granules is seen in cervical smears obtained in conditions of atypical hyperplasia of the cervix. In this condition, varying degrees of dyskaryosis are found as described by Papanicolaou (2). At times, such cytoplasmic granules are seen with atypical hyperplasia in eosinophylic cells without nuclear pyknosis and with one enlarged, usually homogeneous, slightly hyperchromatic nucleus. It is doubtful whether the presence of cytoplasmic granules should be considered a factor in the malignant transformation of cells, in view of the fact that cytoplasmic granules may be found in conditions of increased or prolonged estrogenic stimulation. This is often associated with non-ovulatory cycles of normal or increased length and atypical epithelial hyperplasia of the cervix. It may be speculated that the presence of cytoplasmic granules is a reflection of a possibly carcinogenic hormonal disorder. On the other hand, cytoplasmic granules and globules are often seen in normal squamous cells of buccal smears.

In a series of women treated over prolonged periods with excessive doses of estrogenic hormone, cells with cytoplasmic granules were regularly seen in vaginal smears.

It should be stated that the knowledge regarding the significance of cytoplasmic granules is limited and that further evaluation of this problem by a variety of more extensive methods is desirable.

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DISCUSSION

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

Granulations of the cytoplasm are found in eosinophilic (rarely in cyanophilic) squamous cells with pyknotic or prepyknotic (rarely with vesicular) nuclei in smears of patients whose vaginal epithelium is stimulated markedly by endogenous or exogenous estrogens.

In smears from carcinoma in situ we also found granulations of the cytoplasm in dyskaryotic cells if the normal squamous cells exhibited cytoplasmic granulations.

As far as the cytochemical differentiation of the cytoplasmic granulations is concerned, we can report only negative findings: they are not identical with the sudanophalic granula as described by Ebner (1), nor are they identical with the places of activity of succinic dehydrogenase. Figure 1 demonstrates the appearance of the places of activity of succinic dehydrogenase in a smear from a patient on the 14th day of the menstrual cycle (43 year old patient, unfixed cells, demonstration of the enzymatic activity by precipitation with tetrazolium purple granula). Compared with this, Figure 2 shows the demonstration of succinic dehydrogenase activity in two cancer cells which are similar to basal cells. The localization of the places of enzymatic activity in mitochondria is discussed. It seems that the cytoplasmic granulations are not identical with mitochondria, but this question deserves more extensive investigation.

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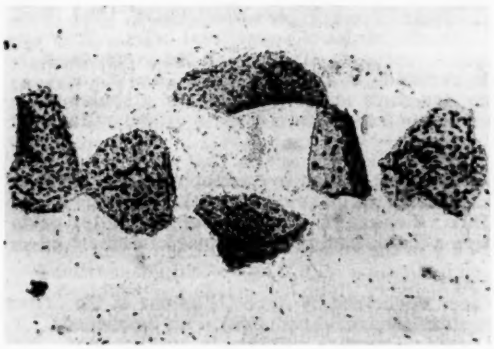

Fig. 1

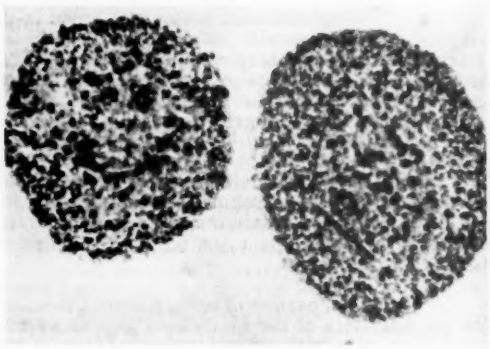

Fig. 2

JEAN DE BRUX, Paris, France:

The cytoplasmic inclusions in the superficial cells under estrogenic effects are always of endogenous origin. The superficial malpighian cells have no macrophagic possibilities.

The granules found in the cytoplasm of the superficial cells have two origins:

(1) Some are from the cytoplasm itself.
(2) Some are from the nucleus.

Granules of cytoplasmic origin are for the most part small and reddish-brown. They express a tendency to "keratinization" of the cytoplasmic components: They are keratohyalin, and the Seligman and Barnett reaction is positive. Hence, we may say that the sulfhydryl and disulfide radicals are abundant. These granules are the same in normal and abnormal dyskaryotic cells.

Cytoplasmic granules of nuclear origin are larger than those of cytoplasmic origin. They stain intense black with hematoxylin, like the pyknotic nucleus in which they have their origin. They have a DNA positive reaction with Brachet's stain. (Some cytologists stain them with Nile blue, Sudan IV and Baker's stain; we have never tried these.)

Hence, we believe that these granules are born from a very fast break-up of the nucleus, by karyorrhexis. We have very rarely found this last type of granule in the abnormal cells.

To summarize, granules originating from chemical transformation of the cytoplasmic components, and granules born from the nucleus are the expressions of a maturation of the cell. This maturation may be induced:

(1) by a fast, excessive and prolonged estrogenic effect on a normal epithelium or

(2) by a dissociated maturation cytoplasm-nucleus--for instance, in dysplasias and in carcinoma in situ.

The granules are the final stage of the normal or abnormal maturation of a malpighian cell.

But by themselves they have no value as a test of malignant transformation.

JACQUES FERIN, Louvain, Belgium:

The cytoplasmic granules can appear fairly rapidly after a few days of estrogenic treatment. For example:

Case 1.: H. R. T. -Pellet of 113 mg. diethylstilbestrol dipropionate. After 8 days, 23% of cells contained cytoplasmic granules.

Case 2.: H. R. T. -5 mg. diethylstilbestrol furoate, intramuscularly. After 7 days, 19% of cells contained cytoplasmic granules.

Case 3.: Secondary amenorrhea-5 mg. estradiol monobenzoate, intramuscularly. After 4 days, 21% of cells contained cytoplasmic granules.

It is possible to find many cells with cytoplasmic granules during estrogenic treatment; we found, for example, 59% in one case.

EMMERICH VON HAAM, Columbus, Ohio, U. S. A.:

Cytoplasmic granules appeared in our experiments on the production of carcinoma of the cervix in mice during the stage known as epithelial dysplasia. They appeared in association with vacuoles and globules and significantly were found not only in the keratinized cells but also in the basal and parabasal cells. They were never considered by us as a factor in the malignant transformation of the cell but interpreted as just one other evidence of disturbed cellular metabolism. I agree with the authors that our knowledge regarding their significance is still too limited to make such far-reaching conclusions as Ayre did when he includes them in his pre-cancer cell complex.

CLOSING REMARKS

HERBERT E. NIEBERGS and HOWARD S. ZUCKER:

No comment.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

CYTOLYSIS AND LONG-TERM ADMINISTRATION OF ESTROGENS

HERBERT E. NIEBURGS
New York, New York, U.S.A.

In the utilization of vaginal smears for hormonal evaluation the cytolytic smear presents certain diagnostic limitations. The cytolytic phenomenon has been studied by numerous investigators and much has been added to the knowledge of cytolysis during the last decade, though certain aspects still remain unsolved. Cytolysis may be due to either the destruction of the cytoplasm by Döderlein bacilli, in which case the term "bacterial cytolysis" has been suggested, or degenerative autolysis of cells without Döderlein bacilli.

Bacterial cytolysis occurs solely in the presence of certain degrees of estrogenic stimulation and affects only one type of cell, which derives from the upper intermediate or lower superficial layer of the epithelium. The nuclei are not affected by the cytolytic process and apparently do not undergo morphological changes. Degenerative autolysis occurs in the absence of Döderlein bacilli and may affect cells derived from the lower epithelial layers.

Stripped nuclei in the absence of Döderlein bacilli are also seen with sub-cylindrical cell proliferation of the endocervix (reserve cell proliferation).

The bacterial cytolysis occurs at a pH level of about 4, while degenerative autolysis takes place at higher pH values.

The manner in which a cytolytic smear develops is not entirely clear. It is not certain as to whether the rapid destruction of the cytoplasm of cells from the upper intermediate layer prevents the maturation of the vaginal epithelium to produce intermediate or superficial cell layers, or whether slightly decreased estrogen production fails to stimulate the vaginal epithelium proliferation above the level of the intermediate layer. The presence of Döderlein bacilli depends on the presence of glycogen in the vaginal epithelium, which in turn results from adequate estrogen activity. The breakdown of glycogen by Döderlein bacilli with the production of lactic acid produces a lower pH which favors the multiplication of Döderlein bacilli. With antibiotic therapy the cytolytic smear may be changed into one presenting cells of intermediate or superficial epithelial cell layers, depending on the degree of estrogen activity. In the author's experience, a slight decrease of estrogen activity, accompanied by reduced epithelial glycogen, is usually associated with a vaginal smear containing upper intermediate cells which often present elongated forms with marked cellular coalescence, which renders the cytoplasmic borders indistinct.

The fact that bacterial cytolysis does not occur in cells below the upper intermediate layer, such as parabasal or lower intermediate cells, is probably due to decreased estrogenic stimulation with decreased epithelial glycogen. The lack of bacterial cytolysis in the presence of superficial cells is probably due to greater resistance of the cytoplasm to the destructive action of Döderlein bacilli in these cells. It is noteworthy to mention that a cytolytic smear is only rarely associated with trichomonas infestation or with a cervical carcinoma.

Incidence of Cytolytic Smears in Physiologic Conditions and Functional Disorders:

Cytolysis is rarely seen during the proliferation phase, at which time, normally, a progressive increase of eosinophilic, superficial cells with pyknotic nuclei occurs. Towards the latter part of the secretory phase, however, varying degrees of cytolysis may be noted. Following menstruation the cytolytic phenomenon of the previous cycle has usually disappeared. During pregnancy, marked cytolysis often occurs and is usually more extensive than when present in the secretory phase. Whether the effect of progesterone on the vaginal epithelium during the secretory

phase and in pregnancy is of significance in regard to the production of cytolysis is not certain. The administration of progesterone to women of childbearing age has failed to produce a statistically significant number of cytolytic smears. On the other hand, the administration of estrogenic compounds resulted in the finding of cytolytic smears in 45% of the women treated, while the remaining 55% of women presented smears with eosinophilic, superficial cells with pyknotic nuclei. The administration of androgen hormones produced a cytolytic smear in an insignificant number of cases. The largest proportion of women under such therapy had vaginal smears presenting eosinophilic, superficial cells with pyknotic nuclei, while a lesser, though significant, number of women had vaginal smears with parabasal and lower intermediate cells. On the other hand Violette Nuovo suggests that the cytolytic smear may be due to slight virilism based on her observation of increased 17-ketosteroids in a large percentage of women with cytolytic smears. In the author's experience, however, hirsutism was only rarely associated with cytolytic smears. The greatest incidence of cytolytic smears was found in women with complaints of dysmenorrhea and premenstrual tension. Menstrual disorders of menorrhagia and hypermenorrhea and amenorrhea were mainly associated with smears free of cytolysis and with varying numbers of eosinophilic, superficial cells with pyknotic nuclei. This is in keeping with Nuovo's observations on the high incidence of cytolytic smears from women presenting premenstrual symptoms and the lowest incidence from women with amenorrhea. On the other hand, in women with abnormal distribution of hair, a significantly high percentage of cytolytic smears was found in Nuovo's series.

In the evaluation of cytolytic smears associated with long-term administration of estrogens, it is essential to consider the dosage of administered estrogenic preparations. In the author's experience no cytolytic smears were noted during the administration of excessively high doses of diethylstilbesterol, from 200-500 mg. daily, over periods extending to several years. The most common findings were smears with predominantly eosinophilic, superficial cells with pyknotic nuclei and anucleate squames. It was also noted that prolonged administration of estrogens is associated with a decrease of the end organ response to estrogenic stimulation.

In order to maintain a high Karyopyknotic Index, the dosage of estrogens has to be increased at certain intervals. The prolonged administration of estrogens in lower dosages commonly employed for the treatment of menopausal symptoms often results in cytolytic smears. This occurs either in women in whom estrogen administration changes a so-called "atrophic" smear with parabasal cells to one with cells of the upper intermediate layers or a regressive phenomenon following the development of a smear consisting of eosinophilic, superficial cells with pyknotic nuclei. The primary development of the cytolytic smear in women on estrogenic therapy may be explained by the occurrence of cytolysis with increase of Döderlein bacilli at a stage at which estrogenic stimulation has produced proliferation of the epithelium to the layer of cells most susceptible to bacterial cytolysis with the simultaneous increase in epithelial glycogen. The secondary cytolytic smear following at varying periods after an initial smear consisting of eosinophilic, superficial cells with pyknotic nuclei may be due to the decrease of epithelial response to estrogens with decrease of epithelial proliferation to the cell layer susceptible to cytolysis. Whether the decrease in estrogenic stimulation on prolonged administration is due to an actual failure of the end organ or to increased inactivation of estrogens in the liver is not certain.

Improved Techniques for Evaluation of Cytolytic Smears under Various Conditions:

Daily washings of the vagina with distilled water throughout the time of estrogen administration or a single intravaginal administration of 100 mg. of Terramycin, Aureomycin, Chloromycetin or other locally effective antibiotic drugs may inhibit cytolysis.

In the author's experience the state of hormonal stimulation in the presence of a cytolytic smear may be adequately evaluated if an additional cervical smear taken from the squamo-columnar junction is examined. The cervical smear is usually not affected by cytolysis and presents a truer indication of the hormonal effect on that part of the epithelium. In such cases in which estrogenic activity is decreased and insufficient to produce epithelial proliferation above the intermediate layer, the cervical smear presents squamous cells of the lower and upper intermediate layers. If, on the other hand, estrogenic stimulation is such that a smear with predominantly eosinophilic, superficial cells with pyknotic nuclei would have developed if not inhibited by cytolysis, the endocervical smear presents eosinophilic, superficial cells with pyknotic nuclei.

The use of this "two smear" method has made it possible to diagnose estrogen and progesterone activity with a high degree of accuracy as judged by correlative studies with endometrial biopsies.

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DISCUSSION

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

One may assume that there is an obligatory participation of the Döderlein's bacilli in cytolysis since cytolysis of glycogen-containing intermediate cells can be discontinued by inhibition of the bacterial growth with antibiotics. In vitro, however, we could not (H. W. Boschann and W. Christiansen) demonstrate this up to now. We added Döderlein's bacilli to glycogen-containing intermediate cells which were obtained from newborn infants or from patients with secondary amenorrhea. The specimen was kept in an incubator and studied microscopically at regular intervals. Definite cytolysis could not be obtained by this means: apparently there are still conditions of the surrounding medium to be explored.

After administration of long-acting estrogens we found the occurrence of cytolysis on the average after the seventh day after the administration. Cytolysis also occurred if there was an atrophic vaginal epithelium and an alkaline pH prior to administration. In some instances one could observe the typical height of estrogenic proliferation only after inhibition of the Döderlein's flora spontaneously occurring with antibiotics. Only then superficial cells with pyknotic nuclei occurred. It seems, therefore, that the Döderlein's bacilli may inhibit the occurrence of the estrogenic height of epithelial proliferation by rapid destruction of the cytoplasm of the cells already in their intermediate stage. When testing long-acting hormones this source of error has to be considered and should be eliminated by local administration of antibiotics. The pyknotic, superficial cells are resistant to the cytolytic action of the Döderlein's bacilli.

One might point out at this occasion that there is a similarity to the histochemical reaction. The positive glycogen reaction (Best Carmin or PAS) in the large parabasal cells and intermediate cells does not appear after pretreatment with diastase, but remains positive in the superficial cells (diastase resistant). The glycogen in the superficial cells seems to occur in such a form which cannot be influenced by diastase and apparently also not by the enzymes of the Döderlein's bacilli.

We had the opportunity of observing cytolysis in dyskaryotic, intermediate cells in cases of carcinoma in situ whereas the more mature dyskaryotic cell forms with pyknotic nuclei were not affected by cytolysis. In invasive carcinoma we could observe cytolysis only very rarely and this only in incipient lesions consisting of relatively glycogen-rich, cytoplasm-rich cells ("middle ripe" forms). Usually one finds, however, that the intercurrent infection causes a change of the pH from acid to alkaline and thus makes it impossible for the Döderlein bacillus to grow and to induce cytolysis.

Approximately 66 per cent of the cases with secondary amenorrhea and postmenopausal women with the intermediate type of proliferation will exhibit cytolysis. Both are conditions in which the participation of the adrenal hormones in the epithelial proliferation is under discussion. When administering long-acting estrogens the slower the decrease of estrogenic action the more often will one find cytolysis (such as observed with estra-diolenanthate or estradol undecylate). Cytolysis persisted until the estrogenic activity decreased to such an extent that there were only parabasal cells present (only containing little glycogen).

VIOLETTE M. NUOVO, Paris, France:

If by "cytolysis" we mean lysis of the cytoplasm by Bacilli vaginalis Döederlein with many free nuclei in evidence in addition to the cellular debris, there cannot be confusion with the term, autolysis.

Under what conditions may cytolysis be found on vaginal smears?

1) Spontaneously, in menstruating women who very often present a modification of the cycle.
2) Secondary to the administration of small doses of androgens to menstruating women.
3) Secondary to the administration of small doses of estrogens to amenorrheic women whose vaginal smears were, previous to treatment, of the pure androgenic type (mainly outer parabasal cells with clear cytoplasm and nuclei).

4) Secondary to the administration of small doses of estrogens given to menopausal women whose vaginal smears were, previous to treatment, of the atrophic type.

Whatever may be the cause of this type of smear, most of the cells are intermediate cells presenting a lysis of the cytoplasm. Since it is practically limited to desquamation of the intermediate zone of the vaginal epithelium, it seems to prove that the superficial zone no longer exists.

This process can be due to:

a) Involution (This would be a case of either spontaneous cytolysis or cytolysis due to light androgenic therapy), or

b) Regeneration (This would be a case of either cytolysis due to light estrogenic therapy in women whose vaginal smear was of the pure androgenic type or of the atrophic type; or due to an abrupt interruption of an important androgenic therapy by the return to normal.)

G. N. Papanicolaou published such a case in 1939 in the March issue of "Endocrinology" under the title "Suppressive Action of Testosterone Propionate on Menstruation and its Effects on Vaginal Smears."

Since cytolysis seems to occur either during involution or regeneration of the vaginal epithelium, the interpretation of the phenomenon may easily be conflicting. Therein may lie the explanation for the conflicting results obtained by various authors. The conflicting results obtained in cytolytic smears by the administration of a certain type of hormone can be explained by the fact that the authors have perhaps not administered it to women having the same hormonal milieu before treatment. For example, for some cytologists cytolysis will not appear after estrogenic therapy, while for others, it will. In this author's opinion, cytolysis cannot appear after estrogenic therapy if the vaginal epithelium was not relatively atrophic prior to treatment, that is to say, if the vaginal smears were not atrophic or a pure androgenic type before estrogenic therapy.

Of course, any pH modification obtained by local treatment can make the cytolysis disappear by changing the vaginal flora, but what seems significant is that it reappears as soon as the local treatment is interrupted.

In connection with Nieburgs' paper, this author would like to know some important features such as:

-- How many patients were treated?

-- Were they menopausal or not?

-- What type of vaginal smears did they have before treatment?

-- Did he administer the same dose of estrogens to all of them and over what length of time?

-- Were there some patients presenting a spontaneous cytolysis and if so were the smears taken regularly twice a week over a period of at least four weeks?

To conclude, this observer thinks that cytolysis is due to a mild hyperandrogenicity as stated in the article Nieburgs referred to. We wish to insist on the fact that those 329 cases studied in that paper were all cases of spontaneous cytolysis:

-- 209 had cytolysis on the smears taken twice a week throughout the cycle.

-- 14 had cytolysis on the smears taken during the follicular phase so the majority of the cases: 67%, presented a cytolysis during the proliferative phase.

-- 94 had cytolysis on the smears taken during the luteal phase.

-- 12 had cytolysis now and then during the cycle.

The physiological and morphological characters that those 329 cases had in common were as follows:

-- long cycles (over 33 days)	40%
-- amenorrhea	23%
-- premenstrual symptoms	84%
-- normal temperature chart	78%
-- hormonal sterility (primary and secondary)	40%
-- one or several miscarriages	45%
-- abnormal distribution of hair	55%
-- high percentage of 17-ketosteroids	57%

Those facts lead this author to think that cytolytic smears could be due to a mild virilism.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

The problem of the cytolytic smears has been studied in a more complete manner by Wied and Christiansen (5, 7), and the findings of these authors have been confirmed and completed by Ost and myself (3). The discussions are too lengthy to be covered in detail here, so I would suggest reading the original papers. Here I will only summarize my own conclusions based upon the findings in more than 1,000 test cases after experimental or therapeutic changes of the vaginal flora or administration of the different sex hormones to castrated and menstruating patients.

First, I would point out that contrary to the opinion of Nieburgs, I am convinced that vaginal glycogen content is independent of estrogenic activity and that cytolysis is not specific for any particular hormonal stimulation. Complete bacteriological, cytological, histological and endocrinological studies have shown that cytolysis is possible only under the two following conditions:

1. presence of a great deal of more or less pure Bacillus Döderlein flora.
2. presence of a vaginal epithelium which has a well developed intermediate layer with rich glycogen content, but which is not covered by a superficial, pyknotic layer whose cells show a decrease in the glycogen content.

Only such an epithelium, which permits a free access of the Bacillus Döderlein to the vaginal glycogen, offers the biologically necessary conditions for the massive growth of this flora and for cytolysis.

Such an epithelium can exist or can be produced by nearly all known hormones which stimulate the vaginal epithelium, especially androgens and progesterone alone or in association with estrogens, while estrogens alone are less convenient since they produce, in general, a more complete vaginal differentiation with rapid formation of a more resistant superficial layer. This explains why the cytolytic smear is rarely found in cases of marked estrogenic stimulation. The exceptions were explained in the above mentioned papers. Table 1 shows the incidence of cytolytic smears in a study done in order to find the relative incidence of cytolysis or Trichomonas infestation in a series of gynecological patients consulting for various disorders or sterility, and where the endometrium (biopsy) could be studied simultaneously the same day. From this table it appears that cytolysis as well as trichomonas infestation (the co-existence of which is really exceptional) can be found associated with very different endometrial pictures, and that cytolysis of vaginal cells is rarely found in true hyperplasia of the endometrium. From the cases of cytolysis with endometrial atrophy it should not be concluded that cytolysis could exist even in vaginal atrophy, since none of these cases showed vaginal atrophy at biopsy. On the contrary, they presented a typical hyperplasia of the intermediate layer, with marked glycogen content, as seen after administration of high dosages of testosterone. The urinary dosages of the 17-ketosteroids proved in these cases the existence of a hyperandrogenic condition. This confirms the findings of Violette Nuovo that cytolysis is relatively frequent in cases of virilism or hyperandrogenism.

Neither the glycogen content of the vagina nor the existence of Döderlein flora can be considered as a criterion for estrogen activity, as proved by several authors. This is important for the interpretation of the pregnancy smears. In my studies so far, I have not been able to find any correlation between the complete cytolytic pregnancy smears and the prevision of the birth of a female, as suggested by Nieburgs. In more than 4,000 cases of pregnancy, the frequency of male and female babies was nearly the same in patients with cytolytic smears. Furthermore, it was found that cytolysis was less frequent during long-term estrogen treatment, either in pregnancy or in non-pregnant women. On the other hand, cytolysis is found relatively frequently in older women with diabetes.

For diagnostic purposes, I would conclude that the cytolytic smear cannot be interpreted and permits no hormonal diagnosis, since the pictures of the cytolytic smears found in the different possible hormonal conditions are identical. One can only exclude the existence of vaginal atrophy. In patients with a cytolytic vaginal smear, cancer of the cervix is unlikely, but this latter conclusion needs confirmation by more extensive studies.

I cannot agree with Nieburgs when he says that in the presence of cytolysis the hormonal state could be adequately evaluated by a simultaneous cervical smear. As shown by other authors and myself, only the lateral cul-de-sac of the vagina should be considered for hormonal smears, since it is the most sensitive part of the vagina for hormonal stimulations, while the squamous cervical epithelium remains inert to even marked hormonal stimulations. In a series (1) of 1,400 cytological tests by simultaneous vaginal and cervical smears in non-pregnant women and in a series of 300 pregnant women who had smears taken from four different sides, the cervical smears gave an incorrect hormonal diagnosis in over 60%. For this reason I believe that a hormonal diagnosis should never be given for a cervical smear or a smear of which the origin is not known. In cases of cytolysis, one can obtain a rather valuable hormonal diagnosis only by two other techniques:

either taking the urinary cytology or after blocking the cytolysis by antibiotics. In the latter case, the possible artifacts produced by such a local treatment and described by Lichtfus and coll. (1) should be well-known in order to avoid other diagnostic errors.

TABLE I

HISTOLOGY OF ENDOMETRIUM	NUMBER OF CASES	CYTOLYSIS	TRICHOMONAS INFESTATION
Complete atrophy	22	3	3
Hypoplasia	71	5	7
Normal proliferation	69	5	3
Moderate hyperplasia	73	0	3
Cystic active hyperplasia	21	0	2
Regressive cystic hyperplasia	8	0	1
Mixed hyperplasia with secretory activity	46	1	3
Insufficient secretory phase (including irregular ripening)	157	31	25
Normal secretory endometrium	104	14	14
Decidua graviditatis	4	0	1
Dystrophic endometrium	15	3	3
TOTALS	590	62 = 10.5%	65 = 11%

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CLOSING REMARKS

HERBERT E. NIEBURGS, New York, New York, U. S. A.:

Pundel's comments are stimulating and it seems apparent that further basic studies on the metabolism of the steroid hormones and their relationship to tissue response is needed. I am in complete agreement with Pundel that cytolysis is not specific. Whether this non-specific response can be extended to "any particular hormonal stimulation," I am not in a position to comment on. I am greatly interested in Pundel's conviction that vaginal glycogen content is independent of estrogen activity, since Pundel confirms that cytolysis occurs solely in the presence of a vaginal epithelium which is limited to the intermediate layer rich in glycogen content. This state of epithelial proliferation requires a certain degree of estrogenic stimulation. Therefore cytolysis is not seen in the presence of marked estrogen deficiency. Conversely, this absence of the epithelial layers above the intermediate zone in the presence of cytolysis does not permit drawing any inference as to the degree of estrogen activity. Not rarely, however, marked cytolysis is found in women with slightly decreased ovarian function. Therefore, in the non-pregnant woman not on hormone therapy, a cytolytic smear excludes marked estrogen deficiency and suggests the possibility of decreased ovarian function. On the other hand, the presence of karyopyknotic cells, often found with cystic glandular hyperplasia, is not necessarily associated with a high estrogenic level but may occur due to prolonged estrogenic stimulation. Hence the change of a cytolytic smear by antibiotic therapy to one with superficial, karyopyknotic cells may not always be an indication of adequate ovarian function.

In pregnancy, however, cytolysis, usually extensive when present, does not appear to have any significance in regard to abnormal hormone levels.

The increased diagnostic accuracy by the use of an additional cervical smear taken from the area of the squamo-columnar junction, in such cases in which the vaginal smear presents cytolysis, has been ascertained in my laboratory over the last ten years. Confirmation of this fact is relatively simple since biopsies taken from the squamo-columnar junction clearly reveal the different state of the squamous epithelium at this site in comparison to the adjacent squamous epithelium.

No reference was made anywhere in my paper to the significance of the cytolytic smear in relation to the fetal sex. Since the early observations made about fourteen years ago, any further work on this subject had to be deferred until completion of studies on more important aspects of cytology.

I am delighted to agree with Violette Nuovo on almost all points regarding cytolysis. I find it however somewhat difficult to accept the idea that androgens are responsible for the destructive activity of the Döderlein bacilli. In our series, the smears following androgenic therapy and in virilism were rarely cytolytic and were mainly associated with karyopyknotic cells.

The women tested in our study were of the postmenopausal and childbearing age. The dosages and type of estrogen compounds varied. The high dosage estrogen administration was carried out in postmenopausal women with inoperable or recurrent carcinoma of the cervix. The statistical evaluation of our findings will be presented in a separate publication.

The comments of Boschann are interesting and partly in keeping with the findings of others and our own. The observation of cytolysis in two-thirds of cases with secondary amenorrhea, in menopause and on prolonged estrogenic therapy is in keeping with decreased estrogen activity and perhaps a reduced estrogen response. Whether any of the adrenal cortical hormones are directly involved in this process is not certain. The occurrence of cytolysis in cases of intermediate cell dyskaryosis, if associated with carcinoma in situ is extremely rare. In my laboratory, in which more than 1,000 cases of carcinoma in situ have been diagnosed, the finding of cytolysis due to Döderlein bacilli is indeed an unusual event even in the cases with intermediate cell dyskaryosis.

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DOES ONE NEED TO GRADUALLY INCREASE THE DOSAGE OF ADMINISTERED ESTROGENS IN PATIENTS UNDER LONG-TERM ESTROGEN THERAPY IN ORDER TO MAINTAIN HIGH PROLIFERATION, AND CAN ONE INDUCE A CONSISTENT INTERMEDIATE TYPE OF PROLIFERATION BY ADMINISTERING LOW DOSAGES OF ESTROGENS?

J. PAUL PUNDEL
Luxembourg, Luxembourg

Since estrogens produce marked vaginal proliferation and progressive differentiation to a completely differentiated superficial layer, one could suppose that by taking the correct dosages of estrogens it should be possible tp produce a consistent intermediate type of proliferation in vaginal smears composed exclusively of intermediate cells.

In 58 castrated or postmenopausal women with complete vaginal atrophy, progressive low dosages of estrogens (estradiol benzoate, diethylstilbestrol, dienestrol, ethynylestradiol) were administered and tested by repeated vaginal smears. The results showed that when the dosage reached the minimal effective level, the vaginal epithelium started to grow. The prior atrophic smear changed progressively to a subatrophic smear (mixture of atrophic or basal and intermediate cells), and then with increase of the dosage, the intermediate cells became the predominant cell elements of the smear. If the estrogens were then continued with the same dosage which had induced the intermediate smear type, one would expect to obtain a persistence of this intermediate smear type. Unfortunately, the control tests did not support this hypothesis. Indeed, the smear continued to change and there appeared superficial cells with pyknosis and even some eosinophilic superficial cells. Even if the estrogens were stopped immediately after the first appearance of the intermediate type, the smear generally continued to show a progressive differentiation to a more superficial type for a few days. After this, it returned progressively to an intermediate type, which persisted for a few days up to several weeks, then to the subatrophic and, finally, to the completely atrophic type of the beginning.

If the estrogens were administered with increasing dosages up to the point where they produce subatrophic smears, and if the increase was then discontinued and the estrogens administered only with the same dosage for several days or weeks, only transitory smear pictures of the intermediate type could be observed.

If the estrogens were administered up to a dosage producing the first intermediate type, then stopped, it was not possible to obtain in every case a consistent intermediate type of proliferation either by giving estrogens with the same dosage or even with a lower dosage from time to time for several days. One observes a continuous up and down of the smears from the intermediate to a more superficial or to a subatrophic type.

Only in 7 cases was a continuous intermediate type of smear observed, with continuous dosage of estrogens above the first minimal "intermediate" dosage; but in these cases cytolysis had appeared, destroying the outer cell layers and thus prohibiting the differentiation to superficial cells.

From these experiences it can be concluded that under normal conditions, especially if cytolysis does not occur, it is very difficult to obtain, with low dosages of estrogens, a consistent intermediate vaginal smear type in the castrated or postmenopausal woman. It seems that the proliferation and especially the differentiation of the vaginal epithelium induced by estrogens has immediately a more complete tendency or that the minimal dosages required to produce karyopyknosis or only intermediate cells are nearly the same. In other words, it seems that if the estrogens are just sufficient to produce an intermediate layer or the first degree of vaginal differentiation, such a vaginal epithelium has immediately the tendency to arrive at a more complete differentiation, as shown by the progressive retraction of the nucleus and the rapid formation of superficial cells, even if the typical intermediate cell layers at biopsy have reached only a moderate thickness. This tendency distinguishes the vaginal effect of estrogens from that of other hormones like testosterone and progesterone, which are able to induce only the first degree of differentiation, or an intermediate layer; and an increase of the dosage does not produce the second degree of differentiation or karyopyknosis and eosinophilia, but only an increase of the thickness of the intermediate layer.

For these reasons, I believe that the continuous spontaneous smears of purely intermediate type which can be observed without cytolysis in cases of amenorrhea or after castration or menopause do not have an exclusive estrogenic origin, but that other hormones interfere, especially androgens, which increase the proliferation and glycogenization induced by estrogens by a synergistic mechanism, but block the differentiation to superficial cells by the specific anti-estrogenic antagonism.

During long-term administration of estrogens, either for castration symptoms or for treatment of endometriosis or cancer metastases, etc., the vaginal smear can present different reactions:

1. With moderate dosages of estrogens, the vaginal epithelium presents rapidly an important estrogen effect with pyknosis up to 90% and eosinophilia to 70% or more. The continuation of these dosages (examples: 10 mg. of estradiol enanthate I. M. every two weeks; 10 mg. of diethylstilbestrol fureate I. M. every two weeks; 2-4 mg. per day per os of diethylstilbestrol or dienestrol) even for months produces identical vaginal reactions. It is not necessary to progressively increase the dosage in order to obtain an identical vaginal reaction. In some cases, especially if the dosage is relatively low (just sufficient to produce an Eosinophilic and Pyknotic Index of about 50% at the beginning) one can observe after a while an increase of these indices, which can be explained by a storage phenomenon of the estrogens in the body. The typical example is presented by castrated patients treated by the implant of a 100 mg. pellet of diethylstilbestrol, for which the daily resorption rate varies between 0.5 and 0.9 mg. 21 patients received such an implant every 8-12 months for over 5 years and the vaginal cytology showed identical reactions after each pellet, as demonstrated in previous papers (1, 2, 3).

In some patients after the menopause or after castration a progressive diminution of the vaginal estrogenic reactions was observed after daily administration of 2 mg. of dienestrol per os. But the vaginal smear then showed a particular cytological picture and control tests of the urinary 17-ketosteroids showed that this decrease of the pyknosis and eosinophilia was possible due to a stimulation of the adrenal production of androgens by the estrogen treatment. The apparent diminution of the estrogen effect in the vaginal smear seems, therefore, not to be related to a lowering of the vaginal sensitivity to estrogens, but caused by the antagonistic effect of the androgens against estrogens.

2. The findings are different if the patients receive high dosages of estrogens, as for example over 15-25 mg. of diethylstilbestrol or dienestrol per os per day over several weeks. In these cases, the vaginal smear at the beginning shows a normal response to estrogens, with a rise of the Pyknotic and Eosinophilic Indices to nearly 100%. But, if the treatment is continued over 1-2 weeks, the vaginal smear shows a progressive decrease of the pyknosis and eosinophilia. For a while this decrease can be stopped by an increase of the estrogen dosage, but finally a further increase of the estrogen dosage has no effect upon the vaginal smear, which tends to a regressive and nearly subatrophic type. The same regressive phenomenon can be observed after massive treatment with androgens or progesterone, and seems to be related to a progressive exhaustion of the vaginal proliferative and maturation potential under extremely high hormonal stimulation.

Conclusions: Under long-term estrogenic treatment, the vagina continues to react normally to administered estrogens as long as the dosage remains within physiological limits. Abnormally high dosages continued over several weeks are followed by a decrease of the vaginal receptivity to estrogens.

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DISCUSSION

JOHN A. FINKBEINER, New York, New York, U.S.A.:

Pundel has written a very excellent summary to these two questions and I am in complete agreement. It is very difficult to obtain a consistent intermediate vaginal cell pattern with continuous administration of low dosages of estrogens. Since the effect of estrogen on the vaginal mucosa is both proliferation and differentiation, it is an unusual experience to be able to separate these effects. Rarely, in an exceptional patient and particularly using the weaker estrogens, one can obtain an intermediate effect for a limited period of time. Much more common is the "continuous up and down" that Pundel describes.

The various types of reactions to long-term estrogen therapy are accurately described. While these are the fundamental and basic patterns, they will vary in different patients with different endocrine and disease status and with different type, dosage, and route of administration of the hormone (viz., local vs. systemic administration).

CLOSING REMARKS

J. PAUL PUNDEL, Luxembourg, Luxembourg:

No comments.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

IS THERE A CONDITION KNOWN OR IS THERE A TIME PERIOD KNOWN IN WHICH THE VAGINAL EPITHELIUM DOES NOT RESPOND WITH MARKED PROLIFERATION TO ADMINISTERED ESTROGEN?

MARCEL GAUDEFROY
Lille, France

This question raises two problems:

1) Are there any cases in which an adequate estrogen administration does not produce proliferation of an atrophic vaginal epithelium? In other words, is there a vaginal estrogenic "non-receptivity"?

Some authors have answered this question in the affirmative. Ferin (1) and Pundel (2) appear to believe that a weak or negative vaginal response is possible, without explaining circumstances of such an abnormality. For my part, I have never observed any atrophic vaginal epithelium which did not exhibit a marked proliferation with an adequate dose of administered estrogen. In this respect, I have studied two cases of double vagina with uterus didelphus. The two half-uteri showed important variations of endometrial pattern from one to the other, but the smears from the two vaginas were identical in both cases. Five mg. of estradiol benzoate given over a period of four days induced an equal or almost equal increase in the Karyopyknotic and Eosinophilic Indices.

I think the problem of vaginal receptivity, if it exists, must be rarer than that of the endometrium.

2) Is there a hormonal condition of genital life in which administered estrogen does not induce proliferation in vaginal epithelium which was not proliferating previously due to the hormonal condition?

Here I can answer more definitely. During normal pregnancy, the vaginal epithelium consists predominantly of intermediate, often navicular cells, and of very few superficial non-cornified cells. It is known that a very high estrogen posology can be given in pregnancy without inducing any colpocytological response (3). Ghilain and Peers have demonstrated (4) that it is necessary to give daily more than 300 mg. of diethylstilbestrol to induce a slight vaginal epithelial proliferation. Personally, I often give pregnant women (with Karyopyknotic Index less than 15% and Eosinophilic Index less than 5%) 2.500 gammas of ethinylestradiol daily without increasing those Indices. I have shown (5) that in particular the failure of the vaginal epithelium to respond in pregnancy to administered estrogens disappears when the ovum dies.

To summarize, there is one period in which the vaginal epithelium does not respond with genuine proliferation (characterized by predominantly superficial cells) to administered estrogens: in normal pregnancy.

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MARIO DE BENNING KAMNITZER
Rio de Janeiro, Brazil

This paper is restricted to observations made in pregnancy and the puerperium.

Using the Papanicolaou staining method and considering as cornified only the karyopyknotic, eosinophilic vaginal cells, we observed that in pregnancy estrogens failed to produce cellular cornification but tended to inhibit pre-existent cornification in cases of so-called "cytologic threatened abortion."

In a group of 20 patients in the second or third month of pregnancy, all of them exhibiting normal vaginal smears, we administered the following estrogenic compounds:

Ethinyl-estradiol	3 mg. daily by mouth
Estradiol Di-propionate	5 mg. in weekly intramuscular injections
Estradiol Mono-benzoate	10 mg. in monthly intramuscular injections

Each of these products was separately tested for its "cornification effect," in periods ranging from 20 to 70 days in the cases treated with ethinyl-estradiol, up to 5 months in the cases who received intramuscular injections. The colpocytologic end result was definitely negative. Similar observations of unresponsiveness to estrogens have been produced with regard to the crystallization phenomenon of the cervical mucus in pregnancy.

In normally proceeding pregnancy the ability of the vaginal epithelium to proliferate under estrogenic stimulation is, however, not impaired. One might ask if, during pregnancy, vaginal cell cornification does not reflect a decrease of estrogenic effect.

In the puerperium, colpocytology allows one to recognise clearly the existence of two distinct biologic phases: a) the genital crisis which begins shortly before the clinical onset of labor (or abortion) and b) the genital recovery, the beginning of which is best defined by the return of normal responsiveness of the vaginal epithelium to estrogens.

In the genital crisis the vaginal smears are atrophic and do not respond at all to administered estrogens, regardless of the given amount of hormone.

Everything points to the fact that the genital crisis is an obligatory episode which cannot be altered or stopped by estrogen treatment before its completion. Estrogens seem, however, to accelerate the course of the genital recovery.

We made, furthermore, a most interesting observation in 8 cases of full term pregnancies, showing that whereas administration of estrogens after delivery failed to produce any recognizable vaginal response, the same hormone if given during the last month of pregnancy did show an unmistakable effect, mitigating the atrophic changes of the smears in the genital crisis and shortening its duration.

Essentially similar observations were also made in cases of prenatal death of the ovum.

The duration of the genital crisis depends mainly on the duration of pregnancy; after full term labor with a living baby it lasts about two weeks. During this time period the vaginal epithelium does not respond with marked proliferation to administered estrogens.

FRANTIŠEK LUKSCH
Prague, Czechoslovakia

Without being able to answer this question directly, I would like to discuss some observations which are closely related to the above question:

In cytological examinations of 30 postmenopausal women with completely atrophic vaginal epithelium who received a single parenteral administration of estrogens (5 mg. diethylstilbestrol or 5 mg. estradiol benzoate in oily solution) we observed two different epithelial reactions:

(1) Normal reaction (complete shift to the right) in which the dosage of the administered estrogen was apparently sufficient to proliferate the initially atrophic epithelium within 6-8 days (maximal values of the Karyopyknotic Index of 25 to 50%) (Fig. 1).

(2) Subnormal reaction (incomplete shift to the right) in which the administered estrogens caused a relative increase in the number of the large, polygonal cells with vesicular nuclei, but which only rarely resulted in the occurrence of superficial cells with pyknotic nuclei (maximal values of the Karyopyknotic Index, 0-4%) (Fig. 2).

This latter reaction occurs less frequently than the former and could be interpreted as a disturbance of receptibility of the vaginal epithelium or as a result of a sub-threshold dosage of estrogens.

Without attempting to evaluate the etiology of this phenomenon, it is recommended that this difference in reaction be kept in mind when considering all those cases in which estrogenic substances do not, or only rarely, influence the Karyopyknotic Index. This is also a reason why we should not attempt to express the effect of estrogens only in degrees of karyopyknosis, especially in those cases in which proliferative changes are expected in deep cellular layers, such as in atrophic vaginal epithelium.

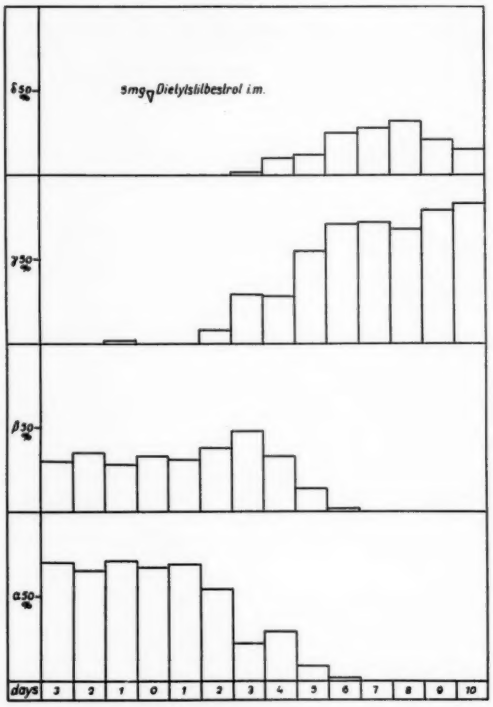

Fig. 1. Normal reaction (complete shift to the right).
alpha - cells of the basal layers
beta - cells of the intermediate layers
gamma - cells of the inner superficial layers
delta - cells of the outer superficial layers
The columns express the percentages.

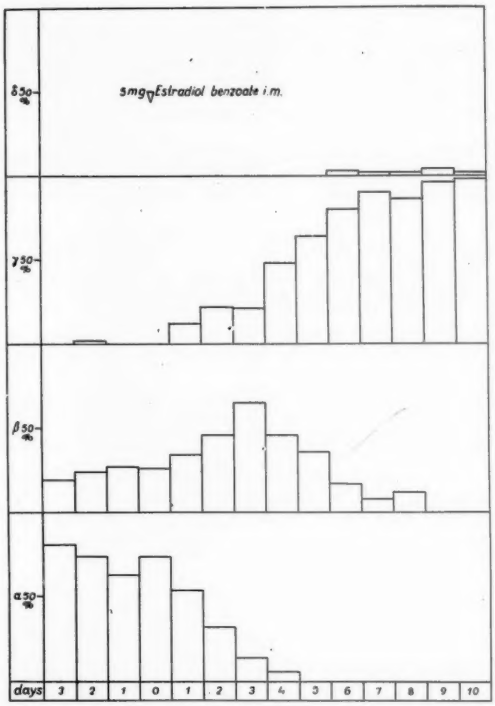

Fig. 2. Subnormal reaction (incomplete shift to the right).

J. PAUL PUNDEL
Luxembourg, Luxembourg

A. PHYSIOLOGICAL CONDITIONS

If we consider exclusively the proliferation of the vaginal epithelium characterized by the increase in the number of cell layers and the number of mitoses at biopsy, I know at the present time only one physiological condition or time period in which the vaginal epithelium does not respond with marked proliferation to administered estrogens: the immediate postpartum period.

If we do not consider the vaginal biopsy, but only the vaginal smear with the specific cytological criteria for estrogen effect as karyopyknosis and eosinophilia, there exist two physiological conditions in which the vaginal cytology does not respond to estrogens with an increase of pyknosis or eosinophilia: the normal pregnancy from the second month to 8 or 10 days before term, and the immediate postpartum period.

1. The Normal Pregnancy: As showed by Van Meensel (6), Gaudefroy (2) and Pundel and Van Meensel (5), the administration of estrogens even in massive dosage (up to 300 mg. of diethylstilbestrol per os per day) does not markedly influence the vaginal smear nor disturb the normal evolution of the pregnancy. The only modifications of the vaginal smear which can be seen during this treatment are a disappearance of the typical clusters, so that the vaginal cells lie more independently, and an occasional rise of the Pyknotic Index without a corresponding eosinophilia. The rise of the Pyknotic Index is most marked in pregnancies before the second month and in the last two or three weeks before term. Only in the first month may the vaginal smear occasionally respond with an increase of the Eosinophilic Index, but only if the estrogens are administered in extremely high dosages. At any other time during pregnancy, a normal and rapid reaction of the vaginal smear to estrogens, as in the non-pregnant woman, has been proven to be a valuable sign of the death of the ovum (1, 3, 5).

But this absence of typical vaginal reactions to administered estrogens should not be considered as a disappearance of the normal sensitivity of the vaginal epithelium to estrogens, for the following reasons:

1. The proliferation of the vaginal epithelium is accentuated, as shown by biopsy. But the vaginal epithelium presents no tendency toward a typical estrogenic differentiation with formation of a superficial pyknotic and eosinophilic layer. It could be explained by the physiological antagonism existing between the estrogens and the progesterone which is produced in marked quantities during pregnancy. The proliferation is accentuated by estrogens, but as the important progesterone stimulation induces a massive desquamation of the outer cell layers, the vaginal cells are shed before they can differentiate completely as superficial cells. But the real mechanism of this absence of estrogenic effect seems to be different.

2. Indeed, as proven by Ghilain and Peers (4) and my own experiences, the absence of specific estrogenic reactions in the vaginal smear during pregnancy exists only for oral or parenteral administration of estrogens, while the local administration of estrogens in the vagina is able in nearly all cases to induce an evident rise in the Pyknotic and Eosinophilic Indices.

As the vaginal smear shows a normal response to administered estrogens if the ovum is dead, one might assume that the orally administered estrogens are transformed directly or indirectly by the placental tissue to progesterone-like substances so that finally they act upon the vaginal epithelium not as estrogens but as progestogens. This hypothesis could also explain why the estrogens administered in high dosage in the first month or shortly before term can induce a certain estrogen effect, because in these cases the hormonal function of the placenta is not yet as marked as in the other months of pregnancy or, shortly before term, is decreasing. This latter decrease before term is markedly accentuated if the pregnancy continues over the term and, in cases of true over-maturity, the estrogens induce a more marked effect than the one observed shortly before the normal term.

For these reasons, it seems that the absence of the typical estrogen effect in the vaginal smear after administration of estrogens during pregnancy should not be considered as a true condition where the vaginal epithelium does not respond at all to estrogens.

2. The Immediate Postpartum Period: Experimental administration of estrogens to patients immediately after delivery has shown that the vaginal epithelium responds neither histologically nor cytologically in a normal manner to estrogens (7, 8). Even with extremely high dosages of estrogens, it was not possible to induce an increase in the Eosinophilic and Pyknotic Indices above a certain level. But this resistance of the vaginal epithelium to estrogens exists only for the first 15 days after the delivery, without any great difference between lactating and non-lactating women. In a series of 100 patients receiving estrogens immediately after delivery in order to block the lactation, I came to the same conclusions: there exists a true vaginal inertia against estrogens

in the first 15 days after delivery. The vaginal smears from patients receiving estrogens and of patients of a control series are practically the same. After the 15th day postpartum, the vaginal epithelium recovers quickly its normal sensitivity for estrogens. The etiological mechanism of this true postpartum inertia of the vaginal epithelium for estrogens is not yet known.

B. PATHOLOGICAL CONDITIONS

It is known that the endometrium can present pathological variations in its sensitivity for estrogens, conditions called by the French authors "metroses de receptivite." As the vagina is another highly sensitive organ for estrogens, I have suggested the possibility that the vagina also could present variations of the normal sensitivity for estrogens, but as far as I know, no proven case of acquired or congenital inertia of the vagina against estrogens has been presented in the literature. In a few personal cases with complete inertia of the endometrium against estrogens (without any possible organic relation), the vaginal smear presented a normal estrogen response. I would like to hear the opinion of other cytologists about this problem.

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DISCUSSION

INES L. C. de ALLENDE, Cordoba, Argentina:

My answer to this question is based on personal knowledge acquired through many years of investigation in the field of colpocytology.

I. Decidedly no epithelia and, therefore, no vaginal smears are refractory or insensible to the action of estrogens.

II. There exists a long period in a woman's life in which the epithelium and vaginal smears do not reveal, with typical proliferation of superficial layers and presence of karyopyknosis and eosinophilia, the estrogenic action exerted on them: normal pregnancy.

III. There exists a short period in the life of a woman in which the epithelium and vaginal smears reveal a delayed estrogenic action exerted on them: immediate postpartum period.

Let us analyze these three assertions.

I

Positive vaginal reaction to administered estrogens, appearance of karyopyknotic, eosinophilic cells, starting from an atrophic epithelium and smear has always been verified:

a) during all the periods in the life of a woman with ovaries present or absent.

b) in sexual anomalies: 1) congenital lack of ovaries and secondary sexual tractus, with or without rudimentary vagina; 2) congenital alterations of secondary sexual tractus: didelphic uterus, with or without double vagina, etc.; 3) male hermaphroditism: embryogenic testis and well-developed vagina with atrophic epithelium (two personal cases).

Conclusions: Any vaginal epithelium, whether atrophic or not, with adequate administered estrogens is able to proliferate and develop the most superficial layers which supply the karyopyknotic, eosinophilic cells to the smear. This vaginal sensitivity to own or administered estrogens contrasts even more in the presence of the concomitant existence of refractory endometria.

II

Facts drawn from observation:

a) In normal pregnancy the karyopyknotic, eosinophilic cells, decreasing progressively generally disappear from the smear after the third month; their absence is prolonged

even after childbirth for a variable period of time according to the different cases.

b) An increase in karyopyknotic, eosinophilic cells during the first three months, or their reappearance in the following months of pregnancy, is frequently found in cases of threatened abortion or fetal death.

Experimental facts:

a) The administration of estrogens—one mg. ethinyl estradiol by mouth, or one mg. dipropionate estradiol, intramuscularly, daily, for 30 days—diminished the cellular grouping and increased the Karyopyknotic and Eosinophilic Indices in the first three months of pregnancy.

Both modifications occurred in a very variable degree in different cases, but in all of them with less marked intensity in relation to the effect that the same dose produces on non-pregnant women.

The same treatment tried in the following months did not modify in any appreciable form the colpocytologic appearance.

b) Under threatened abortion, during the first months of pregnancy when in the colpocytologic series a high percentage of karyopyknotic, eosinophilic cells with an evident decrease of cellular grouping was observed, the administration of progesterone in a high dose modified the vaginal picture, reducing in a remarkable way the Karyopyknotic Index and producing cellular grouping.

Resume:

The facts analyzed, and various others not regarded here, clearly show that: 1) when progesterone reaches a high X level in the organism, the specific signs of estrogenic stimulation disappear in the vaginal smear; 2) if that high X level is maintained, it is very difficult to produce an estrogenic vaginal reaction, even administering high doses of the hormone; 3) if progesterone falls into a lower level momentarily or definitely on account of the death of the egg, the colpocytologic reaction, subject to the influence of the own or administered estrogens, reveals itself in a rapid and intense manner; 4) in this last case, the vaginal reaction to estrogens can be dimnished or even annulled, raising the level of progesterone.

Conclusions:

Vaginal epithelium during pregnancy does not lose its sensitivity to own or administered estrogens. If it does not reveal the existing high estrogenic level, especially during the last month of pregnancy not even with high administered doses, it is probably owing to the interference of the progesterone that masks its specific action on the epithelium and on the smear. This interference is also observed, owing to the inferior hormonal level existing, during the second half of the normal ovulatory cycle. On the other hand, the same interference is exerted on the other structures "target" to sexual hormones: endometrium, endosal pinx, etc., as well as upon the reaction of the cervical mucus crystallization.

What is the intimate mechanism by which administered estrogens during pregnancy reveal their actions?

There exist, among others, three fundamental possibilities: 1) Estrogens find the "effector" modified or insensible to their actions. 2) Estrogens are transformed by progesterone at the moment in which they act on the "effector." 3) Estrogens can act with all their strength on the effector, but their action remains hidden or masked by that of progesterone.

We must remember that the proliferative action of estrogens has a maximum limit, that when reached, successive increases of hormonal level are not further histologically revealed on the epithelium, or, therefore, on the smear.

When both hormones, estrogens and progesterone, are exerting their maximum action, it is useless to raise their level. The progesterone continues exerting a strong exfoliative action, while estrogens rapidly restore the cellular layers which are exfoliated before their complete differentiation.

Other explanations could be found for this hormonal interference; all of them remain in the speculative field since we ignore the intimate mechanism of the tissular action of the sexual hormones, as well as many others.

During the immediate postpartum, the atrophic vaginal epithelium and smear did not respond, in experimental cases, to estrogenic stimulation in the manner and rapidity observed in other stages in the life of a woman in whom the same atrophy exists.

The maximum provoked estrogenic reaction—karypyknosis with eosinophilia—was obtained only on the third postpartum week.

We interpret this "delay" and "sluggishness" with which the vaginal epithelium responds to estrogenic stimulation as a consequence of the exhaustion of the germinal layer, strongly and intensely stimulated during pregnancy.

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

I would like to ask Gaudefroy how the vaginal epithelium of one vagina reacts, in a case of vagina duplex, when estrogen is applied locally to the other vagina.

I would like to ask Gaudefroy and Pundel whether the response of the vaginal epithelium to estrogens during pregnancy is clinically useful as a test for a diagnosis of a dead fetus and whether in their observations, the results of the biological reactions of pregnancy and/or pregnanediol values are known.

I would like to ask Pundel whether the vaginal epithelium is resistant to local estrogen administration in the immediate postpartum period.

The shifting to the right described by Luksch is to be regarded as an effect of the estrogen administration, just as the influence on the Karyopyknotic Index, but less specific, since it can occur also after supply of androgens and progestogens. However, apparently it is useful for the characterization of subliminal estrogen dosages if the influence of other hormones and spontaneous fluctuations in the proliferation height can be excluded.

JACQUES FERIN, Louvain, Belgium:

We have observed some cases other than pregnancy and postpartum in which the vaginal epithelium was suspected to be non-receptive to estrogenic stimulation. For example, a woman surgically castrated many years ago who had never received estrogens was given 0.05 mg. of ethinyl-estradiol a day, orally for 25 days:

the vaginal smear before administration (non-atrophic)

K.I.: 2%, E.I.: 8%

the vaginal smear after administration

K.I.: 0%, E.I.: 4%

We observed, on the other hand, a normal cervical and endometrial response to the estrogens.

JOHN A. FINKBEINER, New York, New York, U.S.A.:

As Pundel has correctly indicated, one must differentiate between proliferation as judged by vaginal biopsy and proliferation as evaluated by the vaginal smear. The vaginal mucosa responds to endocrine stimulation by alterations in proliferation, differentiation and desquamation. Since the chief criterion of estrogenic effect is differentiation, it is extremely difficult to evaluate the degree of proliferation in a highly cornified smear, except for the fact that marked proliferation is implied when such a smear is seen. Since the vaginal mucosa is a target end organ, it can merely reflect the summation of the various stimuli that it receives. Perhaps this explains the difference in the effect of local administration of estrogens indicated by Pundel.

Luksch's observations are confirmed and are most interesting. Liu (Surg., Gynec. & Obst. 105: 241, 1957) has noted this same "subnormal reaction" in some of the patients with advanced mammary cancer being treated with estrogens and has been able to correlate this reaction with failure to show clinical improvement during treatment. Pearson has treated a group of patients with advanced breast cancer who had relapsed following hypophysectomy, with estrogens. None of this group showed objective improvement to estrogen therapy. Despite high dosages of estrogens, the vaginal mucosa often showed little evidence of estrogenic effect.

Therefore, there seem to be a number of physiologic and pathologic conditions in which the vaginal mucosa does not show the usual response to estrogens administered in adequate dosage.

While the type of estrogenic substance, route of administration and dosage are important, there are many more factors that are not known at present.

PIERRE HAOUR, Lyon, France:

As Pundel pointed out in his paper, the two main effects of estrogens on the vaginal epithelium should be considered: proliferation and differentiation (increase in the Pyknotic and Eosinophilic Indices).

If we consider the proliferative effect of estrogens (which is the subject of the paper discussed), it appears that, except in the postpartum period, there are no conditions in which the vagina does not react to estrogens. I believe that any atrophic or non-atrophic epithelium, whether found in childhood, amenorrhea or in the postmenopausal period, can proliferate under estrogen action. I have always noticed a change in smears or vaginal biopsy after a single dose of 5 mg. estradiol dipropionate. We extensively used this test of the 5 mg. injection to clean the cytological picture in cancer diagnosis (1, 2).

Considering the differentiation of superficial layers, I agree that there are conditions in which this effect is not produced by estrogenic action. Pregnancy is one of these conditions, as shown clearly by Gaudefroy, Pundel and de Benning Kamnitzer in their study. In amenorrhea this condition is also encountered and I would like to know the opinion of the authors. The post-menopausal vagina can react more or less to the differentiating effects of estrogens, depending on the type of estrogens and/or the state of the epithelium (atrophic or non-atrophic). There is more or less an increase of the Superficial-cell Index. In 31 women injected with one single dose of 5 mg. estradiol dipropionate, we never observed cases without an appreciable increase of pyknosis and eosinophilia (K.I.: 40-90%; E.I.: 35-75%). The difference with Luksch's results is probably that we did not use the same type of estrogen or the same type of patients (atrophic and also non-atrophic). The estrogen effect of differentiation can be inhibited or reduced by a local antagonism of other steroids or by metabolic conversion of sexual hormones as it has been suggested by Plotz (3).

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BERTHOLD B. HOCHSTAEDT, Haifa, Israel:

Referring to this topic, I would like to quote a certain type of hypothalmic amenorrhea. Over a period of several years we saw eight amenorrheic patients of the mentioned kind (including two cases of Dr. Polishuk, Haifa Governmental Hospital) whose vaginal epithelium did not react at all to repeated attempts with high to extremely high dosages of estrogens given parenterally and/or per os. Cornified cells remained unchanged, 0-4%; uncornified superficial cells, 2-11%. Paschkis and Rakoff sought in such cases anti-estrogenic substances in the blood of the patients (1). Examinations made by us in this direction were negative. Numerous investigations carried out with the aim of clarifying the mechanism of the mentioned phenomena did not yet yield any reproducible results. Further research on the problem is being done at present.

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CAMILLE LICHTFUS, Strasbourg, France:

With our teacher Pundel, we have studied particularly the modifications of the vaginal smear at the end of pregnancy. We injected 20 mg. estradiol monobenzoate (ovocycline) in all women still showing a pregnancy smear type before term. Three days after injection a first control smear exhibited the smear type of a pregnancy at term, if the pregnancy was really at term. In the case of the pregnancy before term, the smear did not undergo any modifications and remained the type as in pregnancy before term.

The test with estradiol monobenzoate separated the pregnancies before term from the pregnancies at term. Moreover, it is the only procedure permitting a precise differential diagnosis between the truly prolonged pregnancy, long-standing grossly abnormal amenorrhea and the pregnancy at term.

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NILO PEREIRA LUZ, Porto Alegro, Brazil:

For better understanding, we could discuss the presented matter on two topics:

1. It is known by all that in pregnancy the administration of estrogens does not influence the Eosinophilic or Karyopyknotic Indices as in non-pregnant women; nevertheless, as was said by Pundel, this is only a part of the subject. The histological picture of the human vagina in pregnancy is that of a highly proliferated epithelium, with more cellular layers than in the non-pregnant vagina. It is quite understandable that an already proliferated epithelium could not grow beyond the potentialities, regardless of the additional quantity of hormones administered. But when one is faced with a disturbed pregnancy with low estrogen secretion, not only does the vaginal smear take on a subatrophic aspect but the picture changes quickly following the administration of the adequate dosage of the hormone (Figs. 1 and 2). It seems we must admit that, if not already proliferated, the vaginal epithelium always responds with marked proliferation to administered estrogens.

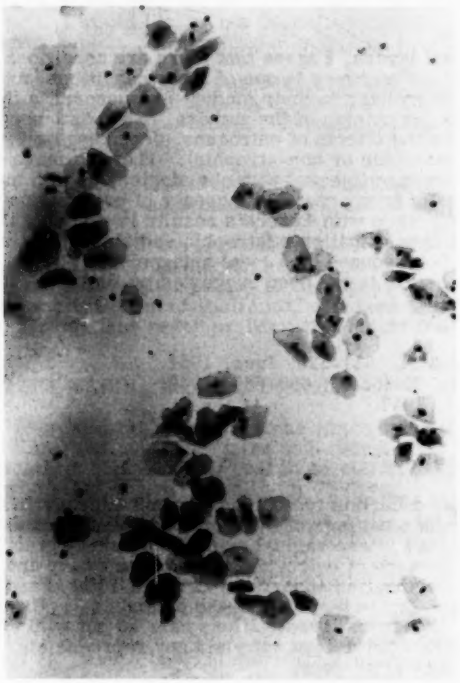

Fig. 1. Low estrogen secretion; low epithelial proliferation; characteristic cytological picture. (Taken on the 30th week - April 23, 1958.)

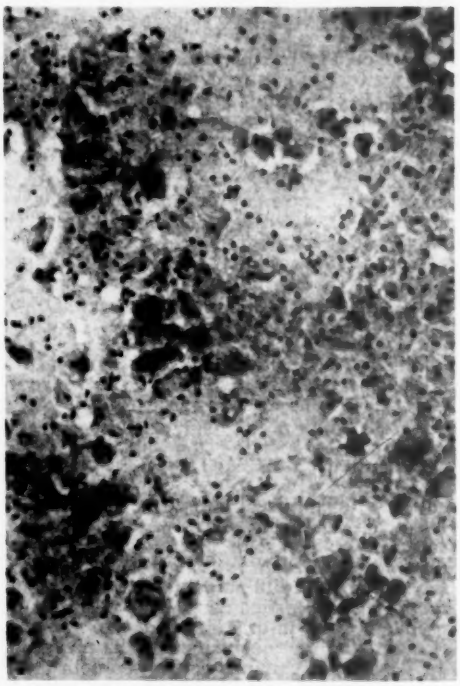

Fig. 2. Same case after weekly microcrystalline suspension of estradiol (10 mg., total dose 30 mg.) plus stilbestrol, 40 mg. daily by mouth. (Taken on May 13, 1958.)

2. Cytologists agree that the immediate post-partum vaginal epithelium remains atrophic no matter how high the doses of estrogens administered, either orally or parenterally. From personal experience I can say also that this is also only one side of the picture since I have verified that the local administration of 0.05 mg. of dienestrol daily induces remarkable proliferative changes that can be cytologically identified from the third post-partum day on; as a rule, the Karyopyknotic Index attains 50% on the fifth day, with 3-5% eosinophilic cells; between the 12th-15th days post-partum, the Karyopyknotic Index is around 60-70% and the Eosinophilic Index between 30-50%. If these findings should be confirmed, it seems that we should consider in the first place the problem of disturbed estrogen metabolism before saying that the post-partum vaginal epithelium is irresponsive to estrogen stimulation for a given period.

LUIS MONTALVO RUIZ, Madrid, Spain:

In this discussion we consider interesting, as does Pundel, the following conditions:

1) the physiological conditions

2) the pathological conditions

1) In the first condition, we find a loss of response to estrogens in the vaginal smears only during a normal pregnancy. The eosinophilic and karyopyknotic cells do not appear except in cases of threatened abortions or premature labor.

Rauscher, injecting up to 5000 mg. of estrogen in ten days, was not able to provoke as a result karyopyknotic, eosinophilic cells after the fourth month of pregnancy.

2) In pathological conditions we have observed two cases of carcinoma of the cervix uteri which were treated with radium producing atrophy of the vaginal epithelium, and which did not respond to estrogens two years after the treatment.

OTAKAR NYKLIČEK, Náchod, Czechoslovakia:

My personal experiences confirm fully the conclusions of main speakers in the respect that only in the period of advanced pregnancy and in the first 10-14 days following the delivery, the vaginal epithelium does not respond with marked proliferation to administered estrogens.

I take the liberty to express, briefly, my opinion about the proliferation of the middle layer of vaginal epithelium in pregnancy and disappearance of the superficial layer, as well as the question why the vaginal epithelium in pregnancy does not respond to the administration of estrogens.

It is known that an immense quantity of progesterone in pregnancy converts the active estradiol and estrone into the slightly active estriol, which may be identified as constituting considerably over 90% in pregnancy. I think that this inactive estriol is not capable of inducing the strongly proliferated middle layer of vaginal epithelium to cornification.

The parenterally or orally administered estrogens are also very quickly inactivated and for that reason cannot show themselves by an otherwise usual vaginal response.

CLOSING REMARKS

MARCEL GAUDEFROY, Lille, France:

I am very happy to see that the authors who have participated in the discussion of this paper unanimously agree that there is an absence of estrogenic effect on the vaginal epithelium during pregnancy. What we mean by "absence of response" should be well understood. We deal with an absence of proliferation of the superficial layer and, as a consequence, with an absence of increase of the Karyopyknotic and Eosinophilic Indices. It is most likely the high production of progesterone, which goes along with a corresponding high level of estrogen and which, during pregnancy, causes the marked growth of the intermediate layers. On the other hand, they cause an abundant and premature exfoliation which inhibits the full differentiation and ripening of the vaginal epithelium.

To the authors who report observations of non-response of the vaginal epithelium after application of estrogens outside of pregnancy or the immediate postpartum period, I must say that I myself have never seen such cases as cited by Drs. Hochstaedt and Ferin.

Finally, to answer the questions put forth by Dr. Boschann, I would like to point out that in the case of double vagina, I have never tested the effect of estrogens by applying the substance to one vagina and looking for the response at the other. If it is possible to see the patient, I will do the experiment and let him know what the results are. As far as the usefulness of vaginal cytology for diagnosing fetal death is concerned, this question will be discussed in detail in Volume III, 1959, Number 1 of Acta Cytologica. The only thing I can say here is that this kind of diagnosis is easy and possible.

FRANTIŠEK LUKSCH, Prague, Czechoslovakia:

According to the extremely interesting panel on this subject, the 3 conditions where estrogen-refractory vaginal epithelium may be found are:

1. after local damage of the vaginal epithelium, e.g., by irradiation (Montalvo): due to peripheral resistancy to estrogen.

2. in hypothalamic-pituitary changes (Hochstaedt, Pearson, cit. after Finkbeiner): due to central resistancy.

3. after castration (Liu, cit. after Finkbeiner) and in the climacterium: due to an indefinite genesis.

All of the above examples indicate that the "resistancy" of the vaginal epithelium during pregnancy takes a separate position and, in these cases, one should talk about "pseudo-resistancy." As shown by the observations of Luz and de Allende, the maximally stimulated epithelium does not lose all of its responsiveness and, therefore, reacts—lower initial degrees of proliferation provided—promptly to the corresponding hormonal stimuli. Thus, we do not deal with a loss of the ability to respond in the actual sense, but, as accurately remarked by de Allende, with a "disguised reactive ability." However, the above listed cases are based upon completely different or even contradictory conditions, i.e., where a more or less resting epithelium reacts either slightly or not at all to hormonal stimuli. Up to the present time, insufficient observations have been made regarding the degree of resistancy. Also, the postpartum vaginal epithelium may fit into this category. Its inability to respond (de Allende) may be traced back to "exhaustion of the germinal layer" which probably could be compared with the above group 1. After these observations and other findings by Haour with "anti-estrogens," it can be concluded: the inability to respond depends to a lesser degree upon the quantity of steroid production than upon unknown shift mechanisms (cellular metabolism vs. mediators) which are put between the hormonal stimuli and the reaction itself. The reference of Finkbeiner on the parallelism of therapy and estrogen resistancy of apparently hormonally influenced breast carcinomas is of special interest.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

I am glad to see that complete agreement exists concerning this very interesting problem which can be summarized as follows:

The vaginal epithelium does not respond in the usual way to estrogens during normal pregnancy from the third month on up to several days before term and also during the first eight to ten days of the postpartum. But during pregnancy this is not a condition of true inertia of the vaginal epithelium against estrogens, since estrogens applied locally induce normal reactions. The absence of typical estrogenic reactions of the vagina during estrogenic treatment administered per os or parenterally can be explained either by the predominance of high quantities of progesterone blocking the normal estrogenic differentiation to karyopyknotic and eosinophilic cells or by a transformation of the administered estrogens in the body to progesterone-like substances. The latter hypothesis seems to be the most acceptable, since in cases of threatened abortion with progesterone insufficiency or in cases showing an abnormal vaginal smear with predominant estrogen effect, the administration of estrogens produces a disappearance of the typical estrogenic reactions in the vaginal smear. The vaginal smear then returns to an entirely normal picture, along with the disappearance of the danger for an abortion. In this case, the paradoxical effect of the estrogens is difficult to explain by a direct interference of high quantities of progesterone, and seems to be related to a transformation of the estrogens to progesterone-like substances. The organ responsible for this change seems to be the placental tissue, for the following reasons: The absence of typical estrogenic reactions in the vaginal smear after estrogen treatment or the paradoxical vaginal reactions to this treatment exist only in normal pregnancies or in disturbed pregnancies which can be returned to normal by treatment; in other words, where the placenta has conserved a rather normal activity.

In the first trimester of pregnancy, estrogens administered in high dosage per os or parenterally can produce in some cases more or less marked estrogenic reactions, which, however, disappear completely after the end of the third month. These estrogenic reactions could be explained by the fact that the placenta, before the third month, is not yet able to transform high quantities of estrogen to progesterone-like substances. In all other cases, and this is the answer to Boschann, a typical estrogenic reaction after the administration of estrogens per os or parenterally is, in my experience, as well as in that of Gaudefroy, a very useful clinical test for the diagnosis of the death of the ovum. However, even in the second month, this test is useful if the dosage of estrogens administered is not too high (25 mg. of diethylstilbestrol per os per day) and if the previous smear showed an abnormal picture. If the estrogens accentuate the abnormal estrogenic smear during the next 48 hours and if this accentuated estrogenic reaction persists over a week, the prognosis is bad, even if the clinical signs of the threatening abortion have completely disappeared. In a total of 103 cases of threatening abortion (from the second to the sixth month of pregnancy) where such an estrogenic reaction was observed, a miscarriage occurred in 102 cases. Histological examination of the placenta and the fetus suggested that the ovum was dead before the beginning of the treatment in these cases. Only in one case did the pregnancy continue up to the eighth month, with a rather small living baby. However, a rather high dosage of estrogens was administered (200 mg. of dienestrol per day per os) for severe bleeding in the second month. This dosage was proved later to be unnecessarily high as, with a lowering of the dosage to 25 mg. per day, the vaginal smear returned to a normal picture. Later, repeated bleeding occurred and was preceded by a rise of the E.P.I. However, an increase of the estrogen dosage immediately produced a return to a normal smear. Unfortunately, I could not obtain in every case hormonal assays or biological reactions, but I can say that in eight cases, the biological reaction in the male frog resulted in a positive estrogen test, and all eight of these cases aborted in the week following the start of the estrogen treatment. The fetus in all eight cases was in a state of advanced decomposition, but in the placenta there remained some areas with a rather active histological picture. (It is well known that in cases of fetal death, biological pregnancy reactions can remain positive up to several weeks after the death.) On the other hand, one should not forget that the death of the fetus,

especially if it happens after the fifth month of pregnancy, does not necessarily induce an immediate abnormal reaction in the vaginal smear, since the placenta can remain active for a time. The negative test was observed also in two cases of bleeding due to a hydatiform mole, with previous highly estrogenic smears. The estrogens produced in 24 hours a return to a completely normal pregnancy smear, but had no effect on the bleeding. I think that this problem will be discussed in a more complete manner in one of the next symposia (cytology of pregnancy).

The observations presented by Pereira Luz concerning the effect of local estrogen treatment in the immediate postpartum are very interesting. Unfortunately, I cannot present personal studies with locally applied estrogens in this period. However, the findings of Pereira Luz seem to indicate that even during the immediate postpartum period, the vaginal inertia against estrogens exists only for estrogens administered per os or parenterally. The mechanism of this particular form of inertia has to be studied.

In my paper, I have not discussed the cases of induced inertia of the vaginal epithelium against estrogens, as after massive local treatment by X-rays or radium. But this inertia appears only if the treatment is massive, while radium treatment with the usual castration dosage (20-27 mcd) has, in general, no effect on the hormonal sensitivity of the vaginal epithelium. This is true even if the treatment has induced a complete and persistent uterine atrophy with secondary inertia of the endometrium for estrogens (1). The progressive inertia of the vaginal epithelium against estrogens which appears during long-term treatment with extraphysiological high dosages has been studied in another part of this symposium.

Concerning the spontaneous pathological conditions of vaginal inertia against estrogens, I have asked in my paper the question: Can there exist in the non-pregnant woman spontaneous vaginal difficulties involving the hormonal sensitivity similar to the well-known difficulties involving the endometrium? The observations presented by Ferin and Hochstaedt now permit a positive answer to this interesting problem. The existence of spontaneous disappearance or non-existence of the estrogen sensitivity in the vaginal epithelium, which in some cases can be completely refractory to any estrogenic stimulation, can now be considered proved. However, it appears that these cases of spontaneous vaginal inertia against estrogens are very rare.

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CAN THE KARYOPYKNOTIC INDEX BE INFLUENCED BY FACTORS OTHER THAN HORMONAL? IF SO, WHAT ARE THEY?

MARCEL GAUDEFROY
Lille, France

Certainly the Karyopyknotic Index can be influenced by non-hormonal factors, which can be frequent causes of mistakes in colpocytological hormonal interpretation. I am referring to the use of vaginal douches or suppositories, anti-inflammatory tablets or jellies, etc., by patients prior to examination. Numerous drugs (frequently, but not invariably) induce an increase of the K.I. and at the same time, of the Eosinophilic Index. In France vaginal douches containing zinc chloride, dioxydimethylmethane disulfonique acid are used. Also iodine tincture, potassium permanganate, or, frequently, tablets of acetarsol are employed. For example, after using a diluted tincture of iodine for 10 days, the K.I. increased from 29% to 68% and began to decrease after 7 days. With diluted zinc chloride the K.I. increased from 3% to 94% in 6 days, and this high value remained for 12 days. With acetarsol tablets, the K.I. rose from 4% to 87% after one month. Often the K.I. and the E.I. increase to about the same extent; sometimes the K.I. alone increases considerably while the E.I. remains practically at the former level.

It is possible to think that the chemical agents produce irritative proliferation with or without keratinization. It is necessary to think about dealing with patients and recommend that they avoid or stop vaginal treatments or toilets for a rather long time, at least 15 days, before any investigation by colpocytology.

I should like to take this opportunity to describe a particular type of smear not infrequently met with: It contains almost exclusively flat, isolated, cyanophilic superficial cells, with a very high K.I. (up to 95-98%) and a very low percentage of eosinophilic cells. The appearance of these smears is dirty, but there is no real mucus and very few polymorphonuclears. The background of the smear is made up of smoothly delicate granular particle (bacterial bodies or cytoplasmic granulation or both), and the cytoplasmic borders are practically lost, without actual cytolysis. This smear type was met with in married or single, pregnant or non-pregnant women. The excretion of some hormones was investigated: 1) FSH: all normal in non-pregnant women; 2) 17-ketosteroids: 9 dosages: 6 scarcely increased, 3 normal; 3) phenolsteroids: 8 dosages: 5 normal, 1 increased, 2 depressed; 4) pregnandiol: 5 dosages: 3 low and 2 high. Endometrial biopsies were as follows: 3 hypoplasia, 1 glandulocystic hyperplasia, 1 normal. Bacteriological identifications: 4 Bacterium coli, 2 staphylococci, 4 enterococci (I think the Anglo-Saxon terminology: the enterococcus streptococcus?), and 5 times among 16 cases, Trichomonas. I believe the etiology of this particular smear type is neither hormonal nor infectious, but what is it? I am wondering if this type of smear is due to the lysis of Trichomonas, and I should be very happy if the eminent discussants would give me their opinions.

ADALBERT MAJEWSKI
Halle a.d. Saale, Germany

In our department, Bernoth and Opitz (1) demonstrated that the amount of the cervical secretion is measurably decreased in patients with hyperkeratosis of the mucosa of the portio (leukoplakia). These results could be proven statistically. In the light of these observations we re-examined the question whether or not, in patients with a decreased cervical secretion, the

Karyopyknotic Index, too, shows any variations. These examinations were undertaken on patients who had been admitted for the exploration of the causes of sterility (cervix factor). In the course of a cycle a smear was taken for a diagnosis of ovarian function, besides the daily measuring of the amount of the cervical secretion. All patients showed a normal biphasic cycle (basal body temperature) and a normal gynecological tactile state.

Figure 1 gives the Karyopyknotic Indices of two patients. Case # 1 shows an average rate of cervical secretion of 0.162 g. with the highest amounts in the ovulatory phase of 0.934 g., whereas the normal average rate runs to about 0.4g. if the cervical secretion is measured daily. The K.I. demonstrates diverging rates in this case. During the beginning of the follicular phase, the number of pyknotic cells was obviously increased. Therefore, the culminating point of ovulation is not necessarily detectable. Still more obviously the deviation of the K.I. is expressed in Case # 2. There the average amount of cervical secretion is 0.0523 g. and is considerably less than in Case # 1. The maximum rate amounts to 0.1302 g. The smear showed almost exclusively cells with very small pyknotic nuclei, also cells without nuclei.

These investigations showed that the number of pyknotic cells in the smear increased with a decrease in the amount of cervical secretion. The K.I. does not vary considerably, insofar as the linear course of the curve is concerned, for highly increased rates. Since the patients showed a normal ovarian function and normal gynecological findings, the hormonal causes for the hyposecretion of the endocervix can be excluded. We must rather suppose a localized dysfunction of the endocervix and the vagina causing too little secretion and insufficient fluid transudation. The insufficient fluid transudation of the vagina results in a higher mechanical stimulation of the mucosa. This may be an explanation for the exfoliation of predominantly pyknotic cells on all days of the cycle.

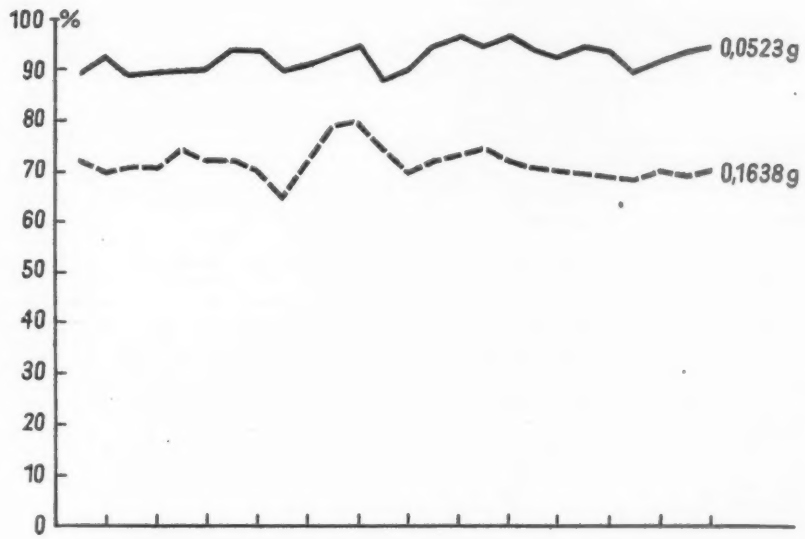

Fig. 1

Figure 1 shows the Karyopyknotic Indices of two patients whose average daily amount of cervical secretion runs to 0.1638 g. and 0.0523 g.

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DISCUSSION

BERTHOLD B. HOCHSTAEDT, Haifa, Israel:

The suggested relationship between karyopyknosis and the amount of cervical secretion is interesting and incites one to further investigation. However, on the basis of BBT and gynecological examination alone, hormonal factors can hardly be excluded in cases like those described by Majewski.

In our cases we met with persistently high cornification curves, closely resembling Majewski's Figure 1; administration of a progesterone-inadequate dosage was followed by a frank depression of cornification with a typical progestational picture. The failure to achieve such a change following administration of progesterone would support the theory of a mechanical stimulation with exclusion of hormonal factors.

To the number of drugs indicated by Gaudefroy may be added diluted lactic acid, frequently used in vaginal douches in our country. Application of this drug was observed to raise the Karyopyknotic Index from 10% to 50-60%.

J. P. PUNDEL, Luxembourg, Luxembourg:

I still believe that the amount of the cervical secretion is not necessarily related to estrogen function or to variations of the Karyopyknotic Index. It depends mostly, in my opinion, upon the anatomical state of the cervix and the amount of cervical glandular mucosa present. In this way, it is evident that patients with a large cervical canal and even with ectopic glandular mucosa can produce a considerable amount of cervical mucus, while in nullipara patients with a cervical stenosis or a rather short cervix the secretion can be very reduced. These anomalies of the cervical secretion can present serious problems in the investigation of sterility cases, but in my opinion, the quantity of the cervical secretion is less important than the quality, which also can present numerous variations, well studied by Palmer. I would ask Majewski if in these cases he has done control investigations by the crystallization test and by bacteriological, cytological and biochemical studies.

I agree with Gaudefroy that the Karyopyknotic Index can be influenced or artificially raised by local irritations of the vagina, even if these alterations are clinically or macroscopically not evident. The most frequent factors are vaginal douches with hygienic lotions or antiseptics, local applications of pharmaceutical products, pessaries, or uterine prolapsus. These possible factors are important to know for hormonal smear diagnosis in routine practice and especially for experimental studies of the particular vaginal reactions of hormones. Frequently, the patients have the desire to appear very clean to the doctor, and before going to a gynecological examination, they take a bath or a vaginal douche, but many women never would confess to the doctor these preparations, and if the doctor does not know much about female psychology, he can look a long time to find out the reason for some unsuspected findings in a vaginal smear. For these reasons, the patients should be instructed very precisely not to take any local treatment, vaginal douche, even with pure water, or have intercourse, not to make any particular cleaning of the vagina or vulva for at least 24 hours before a hormonal smear is to be taken.

The particular smear type mentioned by Gaudefroy is not infrequent. It is found mostly in menopausal women or in patients with estrogen deficiency. This smear type was described at first by Wied, who called it "streptococci smear type." If one finds in a smear a high Karyopyknotic Index without appreciable eosinophilia, it is necessary to study the entire smear picture. If the smear is clean, it permits a valuable hormonal diagnosis. But if the smear presents a dirty, granular ground substance, covering even the vaginal cells, one should use high magnification to look at this granula, which consists of numerous round particles. Bacteriological control studies which I have done of some 100 cases of this smear type showed that these granula are cocci which could be identified as streptococci, Escherichia coli, enterococci, but rarely as staphylococci. Trichomonas vaginalis can be associated, but it is not frequent. It is interesting that leukocytes are rather rare in these smears with sheets of cocci flora. Such a smear should be cleaned up by local treatment with antibiotics before a hormonal diagnosis is made.

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ERICA WACHTEL, London England:

I was suprised to see that both main speakers believe that mechanical factors (variation in cervical secretions and chemical agents) can materially influence the Karyopyknotic Index. In my own experience no such effect has been noted. However, I am convinced that these differences are not based on variations in fact but are rather due to divergences in interpretation.

In severe inflammatory or irritative conditions many exfoliated cells will show pseudopyknotic nuclei, which are a little larger and less dense than true pyknotic nuclei and retain some chromatin structure in contrast to the completely structureless dot the truly pyknotic nucleus represents. It is easy to distinguish between pseudo- and true pyknosis with the help of an oil immersion lens or, as Wied has pointed out, with the aid of a phase contrast microscope.

Pseudopyknosis can be observed in a variety of conditions, e.g., very strikingly in basal cells from smears of patients receiving radiotherapy, in Trichomonas infection, in cells arising from the neighborhood of malignant growths, etc.; but, in my opinion, true nuclear pyknosis is entirely dependent upon the hormonal status of the patient.

CLOSING REMARKS

MARCEL GAUDEFROY, Lille, France:

I agree with Pundel concerning the influence of non-hormonal factors on the Karyopyknotic Index. I am grateful that he insists on advising the patients on precautions before going to the doctor, particularly by emphasizing prohibition of any vaginal douche in order not to change the hormonal evaluation of the smears.

I know that the particular type of smear which I discussed had previously been described by Wied and that he had attributed this type to streptococci. I would like to mention that Pundel speaks of Escherichia coli, among the various microbes in such a smear. Cocci are not in question here, but long shaped bacteria are. I believe that streptococci are not the only bacteria occurring in this type smear and that the exact reason for this smear is not yet understood.

Like Erica Wachtel, I am surprised—but because of her answer. I believe that there is real pyknosis stimulated artificially by non-hormonal medications. Of course, pseudopyknosis is to be observed, but in our papers Pundel and I insisted mainly on an increase of true pyknosis probably secondary to superficial keratinization of the mucosa by chemical means.

Finally, I have to tell Hochstaedt that the drugs specified in my paper are not a limited number, but only some products are able to modify the mucosa. I have pointed out only those which I studied.

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CAN THE KARYOPYKNOTIC INDEX BE INFLUENCED BY HORMONES OTHER THAN ESTROGENS? IF SO, WHICH HORMONES AND TO WHAT EXTENT?

GUILLERMO TERZANO
Buenos Aires, Argentina

The first question may be answered in the affirmative.

It is my opinion that in vaginal smears the Karyopyknotic Index can be influenced by hormones other than estrogens.

The atrophic vaginal smears of castrated women and those of the postmenopausal women (in which all the cells are derived from the stratum spinosum: deep intermediate and parabasal zones) show definite evidence of proliferative changes when progesterone or androgens are administered in adequate doses. (The effect of therapy is checked by serial vaginal smears stained according to Papanicolaou's and Shorr's techniques.)

I. PROGESTERONE ADMINISTRATION

After the administration of progesterone, the vaginal smear (atrophic prior to treatment) changes to a proliferative smear type, with intermediate cells and superficial (karyopyknotic), cyanophilic cells. The scattered eosinophilic cells present are not an indication that progesterone influences proliferation to the eosinophilic, superficial cell level.

Proliferation goes slowly. Appreciable changes do not occur until the patient has received 100 mg. or more of the drug.

The most impressive observation in our series was made with 10 mg. a day in a 74-year-old woman (menopause at 54) in whom a marked increase in the number of intermediate cells started after the sixth injection (60 mg.). Karyopyknotic cells appeared as soon as the patient received the seventh injection (70 mg.). Intermediate cells reached a point as high as 80% with 110 mg. and karyopyknosis, 35% after 170 mg., with no parabasal cells in the smear (Shorr's stain).

Out of the 42 cases in whom we studied the effect of progesterone, 29 (69%) showed a satisfactory response as far as the testing for proliferative stimulation is concerned.

II. ANDROGEN ADMINISTRATION

During androgenic therapy, similar proliferative changes occur, starting with an increase in desquamation (cells in smears are more numerous). Signs of proliferation of the intermediate zone and development of the superficial zone follow. In some cases the vaginal smears of women under androgenic therapy show nothing further than signs of proliferation of the intermediate zone. However, in 129 (79.6%) in our series of 162 observations, superficial cells, most of them cyanophilic, appeared in the vaginal smear. Actual eosinophilic, superficial cells appeared in much lower quantities (never seen higher than 6 or 7%), which from their morphology resembled the superficial cells exfoliated during estrogenic therapy.

The dosage capable of inducing proliferative changes in atrophic vaginal smears varies from one patient to another. Evidence of proliferation has been observed in post-menopausal women with a very small amount of testosterone propionate (15 mg., 5 mg. intramuscularly every two days),

while other women need much larger amounts of the hormone. (This subject has been thoroughly discussed in ACTA CYTOLOGICA Vol. I, 1957.)

As an example of proliferation, I will refer to a 23-year-old woman, castrated because of bilaterally infected ovarian dermoid cysts. Three and a half years later, she received androgenic therapy: testosterone propionate intramuscularly, 10 mg. every two or three days. Prior to treatment: atrophic vaginal smear (deep intermediate cells: 10%, parabasal cells: 90%, no noticeable daily variations). After the sixth injection (60 mg.) there was an evident increase in the number of intermediate cells plus some superficial cells. Remarkable signs of proliferation appeared when the patient had received a total of 80 mg. over a period of about 20 days (Shorr's stain): eosinophilic superficial cells, 3%; cyanophilic superficial cells, 20%; intermediate cells, 77%; parabasal cells, 0; karyopyknosis, 23%.

We did not observe sufficient definitive changes to make conclusions from the smears of castrated or senile women treated with ACTH.

CONCLUSIONS

We consider that androgens and progesterone definitely influence the Karyopyknotic Index of vaginal smears. Nevertheless, the influence is not as great as that produced by estrogens.

In women treated with androgens, some of the intermediate cells and some of the superficial cells have a pale, rather vesicular nucleus. Apart from that (which possibly is due to the androgenic therapy), actual pyknosis can be detected among the "androgenic type cells."

I would also like to add that I refer to "proliferative effect observed in women receiving androgens or progesterone" as a "treatment." The point under discussion now is not whether proliferation is produced by the hormone itself or by some metabolic by-product.

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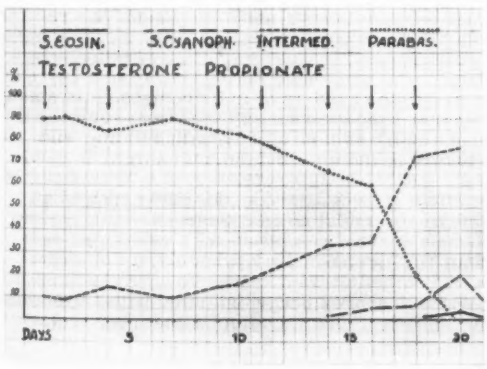

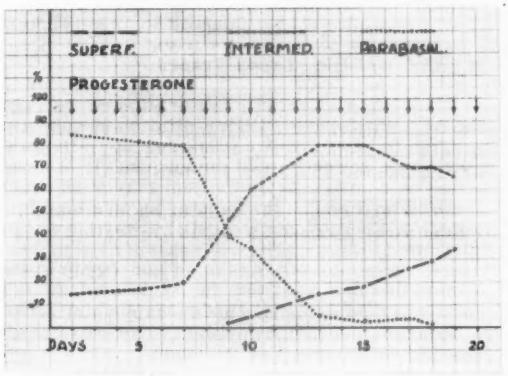

DISCUSSION

H. WERNER BOSCHANN, West-Berlin, Germany:

A 47-year-old female patient, who had an atrophic smear almost one year after menopause, was given 2000 I. U. of serum gonadotropin. Eleven days after the onset of the treatment the cytological picture showed an early follicular phase with a Karyopyknotic Index of 32%. This effect could not be reproduced in women who have been postmenopausal for a longer time than one year.

JEAN de BRUX, Paris, France:

After reading Terzano's paper, I am not convinced that hormones other than estrogens are able to induce true karyopyknosis in cells from the vaginal epithelium. I am afraid the karyopyknotic test used by Terzano is not the one we are generally using in Europe. So I cannot say hormones other than estrogens are able to induce karyopyknosis.

JOHN A. FINKBEINER, New York, New York, U. S. A.:

The whole problem of the cytological effect of administered sex hormones is very difficult and certainly no general agreement seems to exist, as judged by the varying opinions expressed in ACTA CYTOLOGICA, Volume I. There is no question that the Karyopyknotic Index can be influenced by the hormones that Terzano describes. However, I believe that is is stretching a point to infer that these changes are due specifically to the hormone administered rather than to a metabolic by-product. Is it correct to consider solely the Karyopyknotic Index as evidence of activity rather than the effect on the entire vaginal smear pattern? Of course, the question was posed in that terminology, but I strenuously object to focusing one's attention on merely a portion of the vaginal pattern rather than the whole spectrum.

The vaginal mucosa is a target end-organ, not a "cyto-analyzer." Furthermore, the effect of hepatic function, nutrition and the degree of activity and role of various endocrine organs in the metabolism of administered exogenous hormones are not completely understood.

There are far too many unknown factors to make any positive assurances to the question; in our present state of knowledge, I agree with Terzano, but feel that the answer is misleading.

EMMERICH VON HAAM, Columbus, Ohio, U. S. A.:

The author's discussion recalls the presence of highly-estrogenic smears in old women without any evidence of ovarian activity or estrogenic therapy. We now explain those cases as the effect of folliculoid compounds present among the large group of adreno-corticoids. The author's experience with androgens and progesterone seems to make the existence of such folliculoid corticosteroids superfluous.

PIERRE HAOUR, Lyon, France:

The effect of proliferation of the vaginal epithelium is due either to estrogens, progesterone or testosterone. Differentiation of superficial layers, characterized by pyknosis—objectively verified by phase contrast microscopy—is primarily an estrogenic effect. Experimenting with androgenic action in post menopause (1), I never observed pyknosis. But the fact that androgens acting on an atrophic epithelium may sometimes induce epithelial differentiation has been observed in the animal and in the human, as was mentioned previously in ACTA CYTOLOGICA (2). The degree of karyopyknosis is still always low. This estrogenic effect of androgens can be explained in different ways: action of non-purified or particular androgenic compound with estrogenic side effect, metabolic conversion of androgens to estrogens. To speak of true pyknosis, I am of the opinion that one must use the phase contrast microscope; attention should also be given to the possible action of external conditions such as local irritation of the vagina, which may induce pyknosis or increase it if the epithelium is under hormonal stimulation: This fact can be observed in postmenopausal women with prolapse.

Progesterone causes principally an action of desquamation on the vaginal epithelium. This exfoliative process interferes with the vaginal differentiation. Therefore, though I have no systematic experience with progesterone effects upon atrophic epithelium, I believe that progesterone hardly induces pyknosis, or only to a very slight degree. On an epithelium stimulated by estrogens it is clearly shown by the works of Pundel (3) and Wied (4) and the routine examination of smears in the menstrual cycle that pyknosis is reduced by the action of progesterone.

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BERTHOLD B. HOCHSTAEDT, Haifa, Israel:

I agree completely with Terzano with respect to his affirmative answer of the topic question. However, it is to be stressed that the influence of androgens and progesterone is by no means limited to the atrophic vaginal epithelium. As to the effect of progesterone upon the atrophic vaginal epithelium, distinct changes could already be observed after administering 50 mg. of the hormone. In a series of post-menopausal patients with atrophic smears, a very typical cytological picture was observed following injection of progesterone: simultaneous occurrence of eosinophilic superficial cells and isolated parabasal cells. This picture is considered as typical since, following injection of estrogens, the parabasal cells disappear and after injection of androgens generally only proliferation of the intermediate zone appears.

Another observation was made in our experience with androgens. The relative proliferation of the atrophic vaginal epithelium following administration of androgens appears to be directly proportional to a given dosage only up to a certain limit. If androgens are administered in dosages higher than this limit, no further increases, or even decreases of proliferation, could be observed. This occurrence shows that the amount of estrogens which can be metabolized from administered androgens may be limited and therefore, the higher the administered dosage above the limit, the bigger the non-metabolized part and its suppression effect.

LUIS MONTALVO RUIZ, Madrid, Spain:

We are not in absolute agreement with the statements of Terzano, indeed, only in part. The effect of therapeutically administered androgens differs in a castrated woman or in a woman with ovaries, for example: in the menopause. In the first case, proliferation of the vaginal epithelium is produced and in the smears there is an increase in the number of intermediate cells with some superficial precornified cells (Wied and Boschann); in other words, without proceeding to karyopyknosis and eosinophilia.

In the women with ovaries and an atrophic smear, the androgens do not act directly upon the vagina but influence by the ovary through the hypophysis. We now know, through the works of Brown, Husslein and others, that minimal doses of testosterone stimulate the FSH function of the hypophysis and, as a result, produce an estrogen effect of ovarian origin in the vaginal smear. Therefore, the androgen as such does not produce any change in the Karyopyknotic Index. Recently Pundel stated that he has never observed an estrogenic effect following the injection of testosterone.

PROGESTERONE: With the administration of 300 mg. of progesterone we believe it is never possible to obtain karyopyknotic, eosinophilic cells in the vaginal smears of the castrate or menopausal woman.

Pundel and Rauscher are of the same opinion.

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J. PAUL PUNDEL, Luxembourg, Luxembourg:

It is well known that hormones other than estrogens can influence the Karyopyknotic Index in a negative manner. It means that the administration of progesterone or testosterone produces, in women with a high estrogenic or karyopyknotic and eosinophilic vaginal smear, a decrease of these Indices.

For this discussion we have to consider only the contrary influence in a positive manner: If after the administration of androgens or progesterone there appear in the castrated woman superficial vaginal cells characterized by pyknosis of the nucleus (determinated by phase contrast microscopy or exact micrometry), can this pyknosis be considered as a direct effect of androgen or progesterone or not? For the discussion of this problem we have to start from some general considerations:

1. In the woman with completely atrophic vaginal smears, androgens produce a special cytological reaction characterized by the appearance of parabasal or intermediate cyanophilic cells with a particular hypochromatism of the cytoplasm and the nuclei. But in some cases there also can appear superficial cells, mostly cyanophilic. The degree of the Pyknotic and Eosinophilic Indices varies with the type of hormone injected, with the dosage and even with the type of administration. (See the previous symposium.) During the administration of the typical androgens (pure testosterone and chemically pure testosterone propionate or acetate), the appearance of superficial cells is exceptional.

   For technical purposes I would repeat that for hormonal diagnosis the vaginal smears should be taken exclusively from the lateral side of a vaginal cul-de-sac after careful insertion of a speculum. As it is not always possible to avoid completely the introduction into the vagina of cells from the vaginal introitus or even of the vulva (which in general are of the superficial type), the presence in a smear of a few isolated eosinophilic or cyanophilic superficial cells should not be considered as a particular vaginal reaction, but at first as a possible contamination from the outside.

2. Administration of androgens to women showing a marked estrogen smear type produces a decrease of the Karyopyknotic and Eosinophilic Indices and with sufficient dosage the typical estrogen reactions can be completely blocked.

3. The experimental administration of testosterone, progesterone and estrogens has shown that all three hormones have a synergistic proliferative effect upon the vaginal epithelium up to the intermediate layer, but in the further complete differentiation, androgens and progesterone produce an antagonistic effect to estrogens.

4. Patients who are to be chosen for experimental studies for the particular vaginal reactions of a given hormone should be carefully selected. They must present (a) a complete vaginal atrophy, which must be permanent; and (b) all other possibilities able to produce cytological reactions or modifications, such as uterine prolapsus, mechanical, chemical or infectious irritations, have to be eliminated.

5. Before and during the experimental study, complete hormonal assays should be done in order to control the possible reactions of the ovary and adrenals. Even the thyroid gland should not be neglected as we know that the thyroid hormones are necessary to assure the normal sensitivity of the vaginal and endometrial epithelium to sexual hormones.

In his observations Terzano has found rather marked pyknotic and even eosinophilic vaginal reactions after the administration of progesterone or testosterone in his patients with previous atrophic smears, while in my own studies, such reactions are exceptional.

During my experiences with progesterone, I could see that progesterone must be administered in very high dosage in order to produce any appreciable vaginal reactions in the castrated and atrophic woman. With lower dosages (10-20 mg. a day for a week), a marked vaginal proliferation was observed only in patients with previous subatrophic smears or after complementary administration of very small and normally ineffective dosages of estrogens. For the selection of my patients, I have considered as valuable subjects only women with complete atrophic smears (parabasal cells exclusively or with a maximum of 5% intermediate cells), and this vaginal atrophy had to be proven by biopsy and even by a control test of colchicine.

Perhaps the differences in Terzano's findings are due to a different selection or definition of his cases of vaginal atrophy.

In some cases of my studies I also have observed the appearance of a more or less degree of pyknosis or eosinophilia after administration of testosterone or progesterone to atrophic women, but further study of these cases has shown the following conditions:

(a) Some of these women were not correctly selected and further control smears after the test showed that the atrophy was spontaneously variable by temporary rises to subatrophic and even intermediate smear types. In a few patients, urinary estrogen assays showed also a temporary variation from nearly negative results to 20-80 gamma estrone/24 hours.

(b) Some of these patients presented a rise of the urinary estrogen output only during the treatment.

(c) In some of the patients the appearance of pyknotic cells was related to a streptococcoid flora, and treatment by antibiotics was followed by the disappearance of the pyknotic cells.

(d) Some of these patients had received hormonal preparations from a different pharmaceutical laboratory. In three patients my co-workers found that in the same patient the alternative administration of the same dosage of a hormonal preparation coming from two different laboratories produced different vaginal reactions.

From these observations, I would present the following conclusions or suggestions:

In some castrated or postmenopausal women with vaginal atrophy, relatively low dosages of progesterone or testosterone are able to produce some degree of pyknosis or eosinophilia which can be explained by the following possibilities:

1. At least a part of these superficial cells can be artificially introduced to the depth of the vagina from the outside.

2. The patient can continue to produce some endogenous estrogens in a degree which is rather ineffective upon the vaginal epithelium, but with low dosages of progesterone or testosterone one could obtain a synergistic effect between these estrogens and the administered hormones concerning the proliferation, while on the other side these hormones are not yet in a complete antagonistic proportion in order to block a beginning differentiation to pyknotic, superficial cells. Furthermore, individual variations in the antagonistic estrogen-testosterone or progesterone relationship could be possible.

3. The administration of testosterone and possibly progesterone can produce a stimulation of the endogenous estrogen production either in the ovaries or in the adrenals. The latter hypothesis can be based upon the rise of the urinary estrogens found in some cases.

4. During the treatment, especially in young castrated women, there can appear a spontaneous rise of the adrenocortical secretion of estrogens, independent of the treatment, and which has been observed in many cases without any treatment, as proven by repeated vaginal smears or urinary hormonal assays showing that after a temporary vaginal atrophy following the castration, the vaginal smear can spontaneously return to a more or less marked estrogenic type or a more complex type, and that estrogens can be produced again in an important quantity by compensatory secretion by the adrenals.

5. The administered hormonal preparations can contain some estrogen-like impurities or can be transformed in the organism to estrogen-like metabolites. This latter hypothesis seems to be defendable only in very rare cases, since the pyknosis under discussion appears in general after relatively low dosages of testosterone or progesterone, while it is nearly completely absent after high dosage (over 100 mg. per day over a week).

Only if one can completely exclude these suggested possibilities can one conclude that progesterone and testosterone can influence the Pyknotic Index in an estrogen-like manner.

ERICA WACHTEL, London, England:

While I agree with Terzano on the proliferative effect of androgens and progesterones, I do not agree that these hormones in pure form, when given to patients with atrophic smear patterns, will influence the Karyopyknotic Index. Proliferation due to stimulation by androgens or progesterones is mainly an intermediate proliferation, manifest in the smear by intermediate squames replacing parabasal cells, but no significant rise in Karyopyknotic Index occurs.

In my opinion the only influence of hormones other than estrogens on the Karyopyknotic Index is a negative one. Administration of progesterone or androgen to a patient with a high Karyopyknotic Index (due to estrogenic activity) will significantly lower this index.

CLOSING REMARKS

GUILLERMO TERZANO, Buenos Aires, Argentina:

It is obvious that there is controversy, diversity of opinions and disagreement about the subject among cytologists. It seems that the discussions about "Androgens" (1) cannot yet be considered sufficient and further research is needed to clarify whether or not the Karyopyknotic Index can be influenced by hormones other than estrogens.

We cannot continue beating around the bush. Statements such as "testosterone produces proliferation but never pyknosis" and "the appearance of superficial cells is exceptional" do not

seem to fit together. Neither can disagreement be accepted on terminology. I call pyknosis a condensation of the nucleus, with amorphous, darkly stained chromatin, showing no structural details. It is the nucleus of the large, flat, polygonal superficial cells, which (nucleus) is centrally located and is smaller than a red blood cell. Though I did not make my observations under the phase contrast microscope, using Papanicolaou's staining technique, it is not difficult to recognize superficial karyopyknotic cells among the other cells found in the vaginal smear.

It is disappointing indeed that some capable cytologists have observed karyopyknosis (in the above mentioned circumstances) and other capable cytologists have not. (The well known references do not need to be quoted.) It is evident that someone is right and someone is wrong, and there must be, undoubtedly, "a last word" in this. I have described my observations, from which have arisen the interesting discussions of Finkbeiner, Hochstaedt, De Brux, Haour, Von Haam, Pundel and Montalvo-Ruiz, with their good remarks and good points of view. My closing remarks are not destined to settle the problem. The Academy will do it, because the problem deserves further discussion. Meanwhile the discussion should remain open, because ACTA CYTOLOGICA cannot be a simple compilation of points of view and personal experience.

I agree with Finkbeiner that the entire vaginal smear should be considered, and I think that the observation of Hochstaedt is interesting, though we have seen that cytological picture in some post-menopausal women with no treatment at all. But what I would like to say before closing, is that in our cases, the vaginal smears (correctly taken, fixed and stained) were from women who did not have prolapse, infections, irritations or other possible cause of error. All of them had atrophic smears with no daily changes so as to suspect in them a spontaneous proliferation. We have no reasons to doubt that the patients received inadequate progesterone or inadequate testosterone. The cells classified as karyopyknotic were actual superficial cells from the vaginal epithelium (not from some place else) and had pyknotic nuclei. It was not an "interpretation trop simpliste."

My answer "Yes" may be considered as "a little too affirmative" for the question. As a cytologist I am afraid of this, but the real fact is that I have observed signs of proliferation up to the karyopyknotic, superficial cells in the vaginal smears of a relatively large group of our elderly women and castrated women, when we gave them progesterone and testosterone as therapy.

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COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

ORAL AND BUCCAL THRESHOLD DOSAGES OF ADMINISTERED ESTROGENS

GUILLERMO TERZANO
Buenos Aires, Argentina

Since Papanicolaou and Shorr (1936) advocated the use of vaginal smears as "an objective determination index of the effectiveness of estrogenic therapy," it has been generally agreed that "the follicular phase is a reliable standard to test the activity of the estrogenic substances." (It is not necessary to cite articles of the voluminous literature on this subject.)

To find evidence of the activity of the estrogens, vaginal smears of women under therapy were studied for the pattern of Papanicolaou and Shorr's cytological criteria. Qualitative and quantitative evaluations have been made, based on the Karyopyknotic Index and on the Eosinophilic Index.

Large groups of women have been studied in order to balance the presence of highly sensitive and highly refractory individuals. It has been easy, since women complaining of headache, hot flushes, etc., proved to be very cooperative in the control of their treatment and excellent subjects for an experimental research like this.

Estrogens were given in different doses to 10 groups of post-menopausal women (8 to 14 cases each), to 18 castrated women, to 12 severe amenorrheas, and to 22 young girls. All of them had no signs of ovarian activity as demonstrable from the vaginal smears taken prior to the commencement of therapy. The post-menopausal group ranged from 60 to 81 years of age; they were in similar physical conditions and did not have sexual intercourse or vaginal douches, suppositories, etc. during the control.

The potency of the following substances have been tested:

Estradiol Benzoate

Estriol

Diethylstilbestrol

Estrone

Dienoestrol

Ethynilestradiol

Fenociclina

Estradiol-17-beta-cyclopentylpropionate

The threshold dosage has been considered as the minimal amount of estrogenic substances able to produce (in vaginal smears):

(a) some evidence of proliferation up to karypyknotic levels. Still some parabasal cells. (Changes in vaginal smears or threshold of vaginal reaction.)

(b) the pattern of the follicular phase, as during the normal cycle: karyopyknosis (higher) and eosinophilia. No parabasal cells.

The minimal effective doses of estrogens capable to induce in vaginal smears evident signs of stimulated proliferation are as follows:

ORAL THRESHOLD

a) Some evidence of proliferation (one dose):

	Response	
	Minimal	Good
Estrone	0.5 mg	1 mg
Dienoestrol	0.75 mg	1.25 mg
Ethynilestradiol	0.1 mg	0.25 mg
Diethylstilbestrol	0.5 mg	1 mg

b) The pattern of the follicular phase:

	Response	
	Minimal	Good
	Karyopyknosis: 10-30% Eosinophilia: 1-5 %	Karyopyknosis: 30-60% Eosinophilia: 5-25%
Estrone	0.5 mg 5 days	1 mg 5 days
Dienoestrol	0.75 mg 3 days	1.25 mg 4 days
Ethynilestradiol	0.1 mg 4 days	0.5 mg 4 days
Diethylstilbestrol	0.5 mg 3 days	1 mg 3 days
Estriol	– –	1 mg 4 days

BUCCAL THRESHOLD (Sublingually)

a) Some evidence of proliferation:

	Response	
	Minimal	Good
Estrone	2 mg	3.3 mg
Fenociclina	2.5 mg	5 mg
Alfa estradiol	–	0.5 mg

b) The pattern of the follicular phase:

	Response	
	Minimal	Good
	Karyopyknosis: 10-30% Eosinophilia: 1-5 %	Karyopyknosis: 30-60% Eosinophilia: 5-25%
Estrone	2 mg 5 days	6 mg 5 days
Fenociclinica	2.5 mg 6 days	5 mg 7 days
Alfa estradiol	– –	0.5 mg 10 days

DISCUSSION

JACQUES FERIN, Louvain, Belgium:

It is interesting to list the results that we obtained using a surgically castrated woman.

Using the endometrial test (cfr. 2), we have studied the comparative efficiency of various estrogens during treatments of 14 days duration.

Oral threshold dose:

ethinyl-estradiol:	0.01 mg. a day
diethylstilbestrol:	between 0.1 and 0.2 mg.
dienestrol:	between 0.1 and 0.25 mg.
estrone:	0.6 mg.
-estradiol:	1 mg.
methyl-bis-deshydro-doisynolic acid: (Fenocycline)	3 mg.
Estriol:	between 10 and 20 mg.

Buccal threshold dose:

ethinyl-estradiol:	< 0.01 mg.
-estradiol:	< 1 mg.

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ERICA WACHTEL, London, England, U.K.:

While results on parenteral threshold doses of administered estrogens are not yet available from the Hammersmith Hospital, results on oral threshold doses are at hand. The following photomicrographs illustrate a typical response.

Administration of 0.1 mg. stilbestrol daily for 1 week changed an atrophic pattern (with predominance of parabasal cells) into one of intermediate proliferation (predominance of intermediate squames). 0.2 mg. stilbestrol daily for the following week caused the appearance of predominantly cyanophilic, superficial squamous cells without significant rise of the Karyopyknotic Index, whereas a daily dose of 0.3 mg. of the hormone changed the smear appearance into a proliferative pattern. We conclude from these experiments that the oral threshold dose for stilbestrol is 0.25 mg.

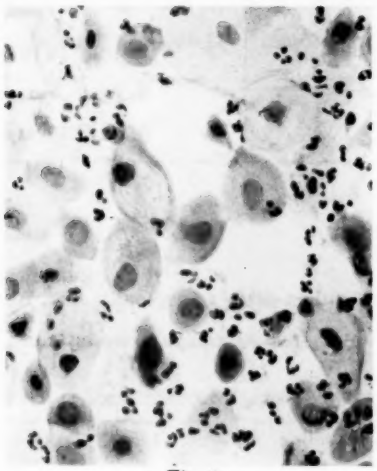

Fig. 1

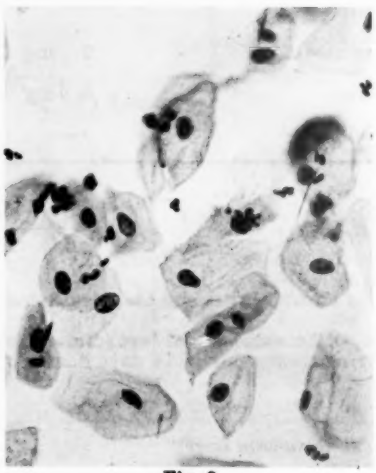

Fig. 2

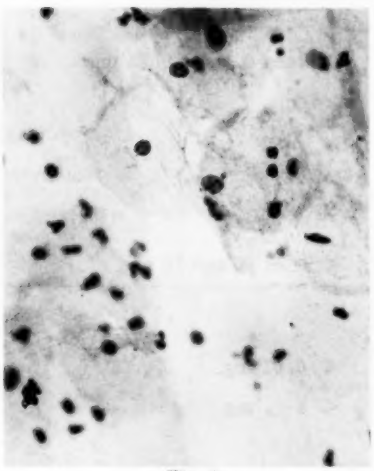

Fig. 3

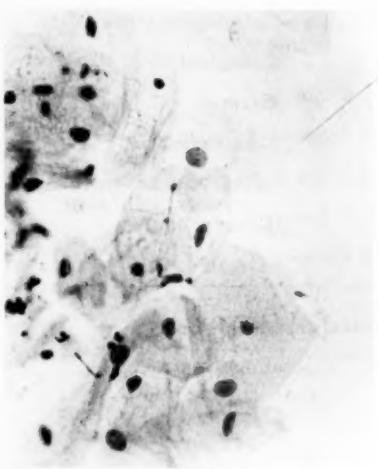

Fig. 4

CLOSING REMARKS

GUILLERMO TERZANO, Buenos Aires, Argentina:

Our figures differ to some extent from those of Erica Wachtel and Ferin. We established the following two threshold dosages:

1. the dosage which induced some evidence of proliferation.
2. the dosage which induced the cytological pattern of the follicular phase.

In our material we arrived at slightly different dosages from those observed by the discussants.

PARENTERAL THRESHOLD DOSAGES OF ADMINISTERED ESTROGENS

GUILLERMO TERZANO
Buenos Aires, Argentina

When estrogens were administered parenterally, the following results have been observed:

a) Some evidence of proliferation. One dose.

	Response	
	Minimal	Good
Estradiol Benzoate		
1) in oil	0.5 mg	1 mg
2) crystals (aqueous sol.)	–	1 mg
Estriol	1 mg	2 mg
Diethylstilbestrol	1.25 mg	5 mg
Estrone (aqueous sol.)	1 mg	2.5 mg
Estradiol-17-beta-cyclopenthyl-propionate	–	5 mg (30 hours)

b) The pattern of the follicular phase:

	Response	
	Minimal	Good
	Karyopyknosis: 10-30% Eosinophilia: 1-5 %	Karyopyknosis: 30-60% Eosinophilia: 5-25%
Estradiol Benzoate		
1) in oil	0.5 mg. 3 days	1 mg. 3 days
2) crystals (aqueous sol.)	– –	1 mg. 4 days
Estriol	1 mg. 5 days	2 mg. 5 days
Diethylstilbestrol	1 mg. 4 days	5 mg. 5 days
Estrone (aqueous sol.)	1 mg. 5 days	2.5 mg. 5 days
Estradiol-17-beta-cyclopenthyl-propionate	5 mg. 48 hours	5 mg. 62 hours

In young girls having vulvitis, the amount of estradiol benzoate (only this estrogen was administered to young girls) necessary to induce a good proliferative effect was much smaller than the dose necessary to induce the same in castrated or post-menopausal women. Since most of the young girls were near their menarche or had infections, they do not seem to be good subjects from which to draw reliable conclusions.

For the vaginal epithelium, good proliferative effect depends more on what kind of estrogen was administered than on the pathway of its administration. Since individual variations in response are not especially remarkable, it seems reasonable to speak of and to test the potency of a given product upon the vaginal epithelium, rather than to speak generally of the reaction of the vaginal epithelium to estrogens.

DISCUSSION

JOHN A. FINKBEINER, New York, New York, U.S.A.:

The threshold dosage of parenterally administered estrogen varies not only with the dosage and type of estrogen and duration of administration, but it also varies from patient to patient. The data that Terzano presents shows the variation that may be expected in dosage and type of estrogen. My experience has been somewhat different, in that free estradiol seems to be about five times as potent as estriol and twice as potent as estrone and stilbestrol, on a mg. for mg. basis. These differences in data may not be important.

However, in my experience, the pathway of administration is extremely important, and the effects of oral, parenteral and local (vaginal) administration are not comparable. Furthermore, individual variations are often marked, particularly in various disease states and endocrinopathies. The response of a group of women with completely atrophic smears who are twenty years postmenopausal is usually more sluggish than that of a group with similar smears who are less than five years postmenopausal, unless both groups have been given a previous "priming dose" and then allowed to return to the completely atrophic pattern. Therefore, Terzano's concluding sentence may not be entirely justified, although it is a reasonable generalization.

LUBA A. TURNBULL, Montreal, Canada:

I have recently had the opportunity of interpreting a hundred vaginal smears from 47 women for estrogenic cornification. These women were 7 years post-menopausal and, for experimental purposes, were using a face cream with estrogenic hormone once a day for 2 weeks.

Prior to the experiment, all the women received a urine test for estrogen with negative results. This work was done in order to find a correlation between estrogen of the urine and the per cent of cornification in smears.

After 2 weeks of daily use of the face cream, the following results were obtained: The vaginal smears of 43 women showed from 3% to 75% of true estrogenic cornification. Smears that had over 15% cornification showed gradual changes to a superficial type of cell smear. Four of the 47 women did not react to the skin cream and continued to show an atrophic smear with 0% estrogen cornification.

CLOSING REMARKS

GUILLERMO TERZANO, Buenos Aires, Argentina:

Qualitative and quantitative evaluations of vaginal smears during estrogenic therapy (the pattern of the smear and the percentage of each type of cell) reveal individual variations.

After having tested the effect of estrogens on the vaginal epithelium of a large group of women, the statement that "the vaginal epthelium is a target end-organ" sounds excellent. Many factors have to be considered. For this reason, the figures of the present report were made from observations on elderly, castrated women with long-lasting amenorrhea, who did not show any evidence (in smears) of ovarian activity, and in whose smears one did not find any disturbing factors which might be potential "sources of error."

It is difficult indeed to establish the actual threshold dosage of administered estrogens. The discussion of Finkbeiner seems to confirm this point of view. I am grateful for his support, and I believe that Finkbeiner would agree that vaginal smears can be considered (for the present) a good qualitative index of estrogenic therapy. It is really important in this phase of endocrinology, and we honestly hope that the time will come when better knowledge will bring about complete agreement. I believe that, based upon further scientific research, the vaginal smear method will prove to be more than "an objective determination index of the effectiveness of estrogenic therapy."

COMMENTS ARE INVITED
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THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

DOES THE INDUCED PROLIFERATION OF THE INITIALLY ATROPHIC EPITHELIUM PERSIST LONGER THAN THE POSSIBLE DURATION OF THE ACTUAL EFFECT OF THE ADMINISTERED ESTROGEN?

GEORGE L. WIED
Chicago, Illinois, U.S.A.

Since exfoliative cytology is used to evaluate the effect and duration of the activity of administered extrogens, this question seems rather important since lack of parallelism in the actual effect and its cytological manifestation would invalidate the cytological procedure of testing estrogenic activity.

Over a period of several years, the author performed cytological examinations on 82 surgical castrates and senile women prior to and after administration of various estrogenic substances in order to test the relative activity of these estrogenic substances. When testing apparently short-acting estrogenic substances, the smears were prepared daily; when testing long-acting agents the smears were prepared every two, three or four days. The Karyopyknotic and Eosinophilic Indices were determined, and cell counts were performed to determine the relative amount of parabasal cells (atrophic), intermediate cells (polygonal, large cells with vesicular nuclei), cyanophilic superficial cells (with pyknotic nuclei) and eosinophilic superficial cells (with pyknotic nuclei). If the values persisted over two or more than two days (within the range of individual variance), they were called "persistent peak values."

The following general observations were made from these studies:

(1) Patients who show an atrophic type of exfoliation on their initial smear sometimes exhibit an entirely different intermediate type of proliferation on subsequent follow-up smears without any apparent cause (no hormonal administration).

(2) The degree of epithelial proliferation in response to administered estrogens varies with the individual patient. One patient with an initially atrophic epithelium will have a Karyopyknotic Index of 15%, while another patient, in response to the same dosage of estrogens over the same period of time, may respond with a Karyopyknotic Index of 35%. However, for evaluation of activity of estrogenic substances, the average values obtained from the examination of 10 surgical castrates or 10 senile women are relatively consistent and reproducible.

(3) Some of the patients who responded in the set time period to the first administration of an estrogenic substance with a Karyopyknotic Index of only 5% responded to the next test with a higher index; whereas in the case of patients who responded to the first test administration with a marked proliferation (e. g., 50% Karyopyknotic Index), the subsequent test administration of the same test substance over the same period of time did not induce any appreciable increase or decrease in the Karyopyknotic Index.

The observations made in our study of 82 surgical castrates and senile women which are specific to this topic are as follows:

(1) In a distinct percentage of test individuals with an initially atrophic epithelium, the proliferated epithelium did not return to the stage of atrophy following a single administration of a short-acting estrogenic substance such as estradiol benzoate or diethylstilbestrol. The observation

period covered more than 24 months. The persistent intermediate cell type consists of rather large, polygonal, cyanophilic cells with non-pyknotic nuclei.

(2) In a distinct percentage of test individuals the atrophic epithelium recurred very promptly a few days after a single administration of estrogenic substance. In a few cases where estradiol benzoate or diethylstilbestrol was administered in single dosages of 5 mg. or 10 mg. each, complete atrophy recurred after 10 to 12 days. Even this seems to be longer than the actual effect of these estrogenic substances is generally considered to last, but bearing in mind the latent period of epithelial reaction, this seems to be the minimal return period to complete atrophy with these substances.

(3) The proliferative effect after a single administration of estrogens varies in intensity and duration with the dosage of estrogens. A single injection of 5 mg. of estradiol benzoate may induce an average Karyopyknotic Index of 15% over an average time period of three days (persistent peak value), whereas a single injection of 30 mg. of estradiol benzoate may induce an average Karyopyknotic Index of 35% over an average time period of 5 to 6 days (persistent peak values). If one considers the individual response and not the average values, then one finds that some patients retain their persistent peak values of the Karyopyknotic Index only for one or two days, whereas other patients retain their persistent peak values over four or five days after a single administration of 5 mg. of estradiol benzoate.

(4) When long-acting estrogenic substances were used such as estradiol valerate, estradiol cyclopentylpropionate or estradiol undecylate, the above observed differences in epithelial response became even more apparent. In some cases where 30 mg. of estradiol undecylate were administered in a single dose, the induced increase in the Karyopyknotic and Eosinophilic Indices lasted over 45 days, whereas in others the same dose induced the persistent peak values only over a period of from eight to nine days. Practically none of the patients tested with this particular substance returned to complete atrophy in an observation period of up to two years. The persisting cell type consisted of large, polygonal, cyanophilic cells with nonpyknotic nuclei (intermediate cells) but has been definitely different from the initial atrophic type prior to administration of the estrogenic substance.

In answer to the topic question one may state:

(a) Induced proliferation of the initially atrophic vaginal epithelium may persist considerably longer than the actual effect of the administered estrogen if one were to use the disappearance of the so-called atrophic cells (parabasal cells) and the appearance of large, polygonal, cyanophilic cells with vesicular nuclei (intermediate cells) as criteria for proliferation. In some cases the initially complete atrophic stage has not been reverted to, even after observation periods of several years. The evaluation of the return period to complete epithelial atrophy should, therefore, be disregarded in hormonal tests for the evaluation of duration and efficiency of administered estrogens.

(b) Induced proliferation of the initially atrophic vaginal epithelium varies with individual patients and with the dosage of administered estrogens if the Karyopyknotic Index is used as an indicator of proliferation, but the induced average results stay generally within the relative range of the possible actual effects of administered estrogens (considering the epithelial latent reaction).

(c) Induced proliferation of an initially atrophic vaginal epithelium may be estimated rather accurately if one determines the induced result on values for 10 patients by establishing the average persistent peak values of the Karyopyknotic and Eosinophilic Indices. Using these average values as indicators, the induced proliferation of an initially atrophic epithelium does not persist long (considering the epithelial latent period) as compared with the probable actual effect of the administered estrogen.

(d) The question why, in some cases, is the initial stage of epithelial atrophy not reached for an overly long time, deserves further investigation. One might also include studying the roles of the connective tissue and the adrenals in this phenomenon.

DISCUSSION

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

Based on experiences made during test examinations of various hormones on the vaginal epithelium, I can fully agree with the findings of Wied.

One often observes spontaneous changes in the height of proliferation in women with atrophic vaginal epithelium. This is less important for the studies on estrogens than for those performed on androgens and progestogens. After administration of the latter two substances, even in high dosages, one will find that there were only some intermediate cells exfoliated in addition to the originally present parabasal cells. In these cases there is always a possibility that the slight changes mentioned above were a result of spontaneous proliferation. Therefore, we consider only

those individuals suitable for androgen and progestogen tests whose vaginal smears exhibit a persistent atrophic type, which has to be ascertained in serial examinations prior to the test. The epithelial proliferation reaches to the superficial cell layer with pyknotic nuclei after administration of estrogens. This height of proliferation cannot be observed after spontaneous proliferation. One can, therefore, neglect the possibility of spontaneous proliferations when studying the effects of estrogens on the vaginal epithelium. This is one of the reasons why estrogens can be tested more accurately on the vaginal epithelium than the other ovarian hormones.

The individual variance in epithelial response is greater when the effectiveness of the hormonal substance is less. Uniform results indicate marked proliferative activity. As a matter of fact, one may say that considerable variance in individual response is indicative of slight activity. One observes also that individual results vary more often after administration of long-acting substances than after the administration of short-acting agents. Individually different resorption, storage and metabolism of the substance, factors which are not related to the vaginal epithelium, may also play a role in these cases.

The average persistent peak value is by far the most reliable criterion for the duration of activity of the hormonal substance. The persistent peak value is less influenced by individual differences in response than is the variable regression period to the original height of proliferation. This can be extremely variable after administration of long-acting substances. It is not known why the enzymatic system of the proliferative epithelial zone is stimulated more markedly in some cases than in others after administration of equal dosages of the same substance. Possible individual retroactions of the adrenal cortex are not fully understood either. It should also be mentioned that there is a distinct latent period between the hormonal stimulus and the morphological response. This latent period is found either when the hormonal level is rising or falling. In certain cases one can observe that the height of epithelial proliferation is still increasing while the hormonal excretion studies show clearly that the actual substance is present only in considerably decreased quantities. One observes estrogen withdrawal bleeding on the fourth or fifth day after injection of estradiol benzoate, indicating a decrease of the estrogenic activity below a distinct threshold dosage; whereas one observes the maximal height of vaginal epithelial proliferation only around the seventh day after administration. However, this latent period may be neglected in the tests because it is observed consistently in each individual. The height of proliferation represents, then, a true hormonal effect even when the actual substance is already metabolized and excreted. Similarly, from the presence of a glandular hyperplasia of the endometrium one can deduct that estrogenic effects must have persisted over a long period of time. This is true even if there is already uterine bleeding present as a result of relative estrogen deficiency and if, also, the vaginal epithelium already shows the criteria of estrogen deficiency (due to reacting faster than the endometrium).

Despite all of these limitations which merely show the relative value of cytological testing of hormonal substances, one may say that the vaginal epithelium gives us information on: (1) whether or not a substance has estrogenic activity, (2) whether one is dealing with a long-acting or short-acting substance and (3) whether or not the estrogenic substance under investigation is more active than previously tested estrogenic substance. The average persistent peak value represents the practical useful indicator for the duration of the effect. The complete regression to the atrophic type, however, is for some unknown reasons subject to such great individual variances that it should not be used as an indicator in the evaluation of the duration of the hormonal effect.

BERTHOLD B. HOCHSTAEDT, Haifa, Israel:

The very instructive general and specific observations of Wied give rise to many further problems as to the approach and evaluation of cytological examinations. In the light of these observations a question of utmost practical importance has to be considered anew: if a patient received estrogenic treatment, how much time has to elapse until a reliable cytohormonal examination for diagnostic purposes may be performed?

In order to determine the duration of effectiveness of some estrogenic substances, their suppressing effect on the increased urinary FSH excretion is castrated and postmenopausal women was studied (1). Simultaneously serial vaginal smears were examined. In the majority of cases the induced proliferation of the vaginal epithelium persisted 5-18 days (in some patients until 30 days and in a few cases up to several months) longer than the suppressing effect on the FSH excretion. The duration of the clinical effects (disappearance of vegetative and vasomotor disturbances) in one group of the cases corresponded with the action upon the FSH level and in the second part paralleled the effect on the vaginal epithelium. Now the question arises: which one of the mentioned effects is to be considered as the "actual one"? Besides their action on the genital tract, estrogens are known to influence in a varying extent all the systems of the human organism. Therefore, one "actual effect" can scarcely be talked about, as it is in the topic question itself.

The cause of the prolonged vaginal effect may be related, it seems to me, to a stimulation of endogenous estrogen production (e. g., adrenals) brought about by the administered dose

of estrogen. Results of quantitative determinations of estrogen in blood and urine, carried out in the mentioned patients, support this hypothesis. However, another possible explanation could be made on the basis of Selye's "pituitary shift": following inhibition of FSH, secretion of ACTH and adrenal steroids is increased. Some of the adrenal hormones, e.g., androgens, are partially converted by demethylation and aromatization to estrogenic substances, which later may be the cause of the prolonged proliferation.

Bibliography

1. Hochstaedt, B.B. and Langer, G.: Long Termed Treatment of the Castration and Climacteric Syndrome, Harefua, 1958.

CLOSING REMARKS

GEORGE L. WIED, Chicago, Illinois, U.S.A.:

I would like to thank both discussants, Boschann and Hochstaedt, for their extremely stimulating contributions.

The basic questions raised by Hochstaedt are of great interest and deserve careful consideration. With Boschann, too, I am in full agreement. He has supplemented my presentation with some important details which show the limitations of this technique. However, I would like to add that despite these limitations, the cytological technique of determining relative estrogenic activity is (for clinical use) the most useful and least expensive one known at this time. If properly evaluated, the cytological specimens provide an accurate test as to the duration of the effect of the estrogenic activity.

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WHAT ARE THE RELATIVE DOSAGES OF ANDROGENS PLUS ESTROGENS WHICH SUPPRESS THE OCCURRENCE OF THE HIGHLY PROLIFERATED CELL TYPE?

HANNS-WERNER BOSCHANN
West-Berlin, Germany

For the answer to this question, conclusions were reached by studies concerning the behavior of the atrophic menopausal epithelium after supplies of estrogens, androgens (those which are adequate in strength and duration of effect) and their mixture. Because of the uncertain factors connected with every peroral therapy, chiefly results of the parenteral applications were used. The Karyopyknotic Index was chosen as a criterion of the proliferation height. Only those nuclei were interpreted as pyknotic which appeared light red in phase contrast observation if stained after Papanicolaou (G. L. Wied, 1953, 1955). In this way, the exact count was facilitated by elimination of subjective interpretations of "pyknotic."

I. Androgens alone stimulate the vaginal epithelium to a medium proliferation height. Predominantly cells of the intermediate layer and also cells with vesicular nuclei from the superficial layer result. With weak androgens or insufficient dosages one finds the parabasal and deep intermediate cells even at the height of the effect. One finds superficial cells with pyknotic nuclei--depending on the kind and dose of the acting androgens--between 0 and 20% (cf. H. W. Boschann, ACTA CYTOLOGICA 1: 87-89, 1957.

II. Estrogens alone stimulate the atrophic vaginal epithelium to the highest possible proliferation. In the smear one finds numerous superficial cells with pyknotic nuclei-depending on the kind and dosage, in 30-90% and more.

III. Estrogens and androgens simultaneously will give the following results on the vaginal epithelium:

1. a (quantitative) synergism
   a. relative to the intensity of the proliferation (recognizable by the number of cells)
   b. independent of the ratio of androgen to estrogen.
2. a (qualitative) antagonism
   a. relative to the height of the proliferation (recognizable by the Karyopyknotic Index)
   b. dependent on the ratio of androgen to estrogen. (Sit venia repetition!!)

The twenty-fold dose of androgens can block the specific estrogen proliferation at the proliferation height which the androgen alone would attain.

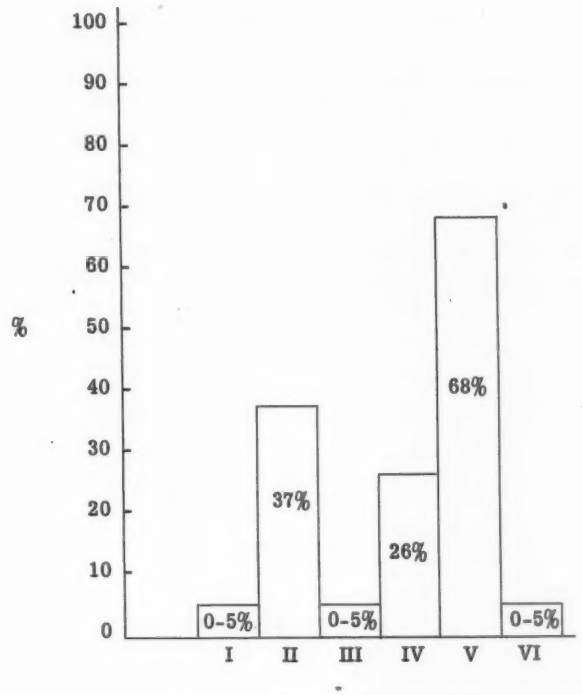
With increasing estrogen or decreasing androgen, thus with a shifting of the androgen: estrogen ratio (1:20 in favor of estrogen), the estrogen influence dominates more and more. With the ratio of one part estrogen to ten parts androgen, the picture is generally clearly estrogenic.

These considerations come from the following experimental results:

Table I

Estradiol valerate	Testosterone enanthate			
	0 mg.	50 mg.	100 mg.	250 mg.
0		0-5%	0-5%	0-5%
1.0 mg.	10-30%	0-5%		
3.0 mg.	30-40%	5-20%		
4.0 mg.	30-50%		0-5%	
6.0 mg.	30-60%		5-20%	
10.0 mg.	more than 50%		more than 50%	0-5%
% = Karyopyknotic Index				

Table 2



This local antagonism of androgens and estrogens in relation to the proliferation height gives the explanation for the influence of androgens on the smear picture of the woman in cycle. Very large androgen dosages must be given in the cycle in order to block, in the twenty-fold dose, the adundant, present; endogenous estrogens, and therefore, to discernibly decrease the proliferation height. These doses (3-500 mg. in a month) are, however, so large that at the same time they may decrease the FSH secretion of the hypophysis and therefore local antagonism and central suppression of estrogen production cannot be differentiated.

It is stated in the literature that the necessary amount of androgen had to be up to 250 times larger than the amount of estrogen. This is explained by the different severities and dura-

tions of the effects of various androgens and estrogens: furthermore, that instead of the parenteral supply one or both substances were administered buccally or perorally. When androgens are not given parenterally, it is noticed that for equal effects on the vaginal epithelium the buccal dosage must be approximately six times as large and the peroral dosage twelve times as large as the parenteral amount. Individual variations are to be expected more frequently. For this reason a ratio good for all combination and application types is not possible. The ratio of twenty parts androgen to one part estrogen is useful for the therapeutic, common esters of testosterone and of estradiol in parenteral application. There is a general significance insofar as the incidental androgens and estrogens can easily be brought in relation to these compounds and that in this way the presumable behaviour of the compounds can be derived in advance, even if different kinds of application are used.

DISCUSSION

JACQUES FERIN, Louvain, Belgium:

We have observed three surgically castrated women and obtained the following results:

RESULTS OF DAILY HORMONAL THERAPY

Ethinyl-Estradiol	+	Methyl-Testosterone	=	Smear Type
0.025 mg.		50 mg.		Estrogenic
0.05 mg.		50 mg.		Estrogenic
0.1 mg.		50 mg.		Estrogenic
0.1 mg.		100 mg.		no superficial cells
0.25 mg.		100 mg.		no superficial cells
0.5 mg.		100 mg.		no superficial cells

Both steroids were given sublingually simultaneously for 20 days and a smear was taken at the end of treatment.

We have been asking the question whether it wouldn't be possible that once the methyltestosterone reaches a certain level, the estrogens, whatever their dosage, cannot exercise their specific action on the vaginal smear any more.

PIERRE HAOUR, Lyon, France:

This new and interesting development of Boschann's studies on the action of androgens on the vaginal epithelium shows again that these hormones can induce proliferation, but are principally antagonists of the differentiation effect (measured by the degree of karyopyknosis) induced by estrogens. Androgens may yet produce a certain degree of karyopyknosis by themselves (which in Boschann's paper does not exceed 5%, even with increasing dosages).

The figures given by Boschann provide a very useful basis for studying the activity of different types of estrogens and androgens.

We would greatly appreciate if the author could give any precise information as to a clinical application. Is there any optimum clinical effect which can be correlated to the vaginal response? The only experience I have with the association of androgen and estrogen is with testosterone enanthate and with estradiol valerate (90 mg. plus 4 mg.). Pyknosis appears eight days after the injection; clinical symptoms are usually relieved at that time; even with repeated injections, pyknosis does not exceed 5%.

Boschann stresses that androgens can depress FSH in the menstrual cycle. Are there any data proving this effect in the normal menstrual cycle? With the usual procedure of assay I believe it is difficult to obtain, even with large doses of androgens, a depressing effect of FSH under the normal level; in the menstruating woman, except near the menopause, FSH is always found in the normal ranges.

BERTHOLD B. HOCHSTAEDT, Haifa, Israel:

The answer of Boschann to the question is of such a precise and sharply defined nature that one hardly could imagine a better one in medicine and biology. I would only like to report an observation which is connected with the above question. In order not to intensify any virilization which is observed in a distinct portion of the climacteric patients, we have tested 25 women patients to discover the minimal amount of testosterone which might still suppress the undesirably high proliferation, but which is still sufficient together with the administered estrogen to inhibit the increased FSH production. In cases with increased excretion of 17-keto steroids (more than 20 mg pro die) we could observe that the specific estrogenic proliferation may be suppressed with a dosage of testosterone proprionate which is 11 to 14 times higher than the estrogen dosage. Our results confirm the experiences of Boschann as far as the patients with normal 17-keto steroid excretions are concerned. Our clinical material on which we base this observation is too small to be evaluated statistically. It would be of general interest to hear if Boschann has made similar observations.

CLOSING REMARKS

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

I would like to thank the discussants very much for their suggestions which increase the knowledge of this subject.

To Dr. Ferin:

My co-worker, Dr. S. Kur and I applied Dr. Ferin's method on women with atrophic vaginal epithelium. For example:

patient F,	65 years old,	20 years post-menopausal,	atrophic smear.
patient L,	56 years old,	8 years post-menopausal,	atrophic smear.

Both received 10 mg ethinyl-estradiol and 100 mg methyl testosterone sublingually. Results:

			Patient F	Patient L
2 days after start of treatment:	Karyopyknotic Index	-	0:100	0:100
4 days after start of treatment:	Karyopyknotic Index	-	19: 81	10: 90
6 days after start of treatment:	Karyopyknotic Index	-	24: 76	12: 88
8 days after start of treatment:	Karyopyknotic Index	-		14: 86
10 days after start of treatment:	Karyopyknotic Index	-		20: 80

Then both women received 10 mg estradiol benzoate i. m., while sublingually the daily 100 mg of methyl testosterone was continued. Results:

			Patient F	Patient L
4 days after injection:	Karyopyknotic Index	-	41: 59	39: 61
8 days after injection:	Karyopyknotic Index	-	44: 56	17: 83

On the basis of these results, we must give a negative answer to Ferin's question -- "Whether it would not be possible that once the methyl testosterone reaches a certain level, the estrogens, whatever their dosage, cannot exercise their specific action on the vaginal smear any more?" The most important factor appears to be a sufficient resorption of estrogen.

To Dr. Haour:

The clinical importance of the vaginal smear in the use of androgen-estrogen mixture consists in showing:

1) whether the estrogen component has not been overdosed (in this case the Karyopyknotic Index would exceed 20%)
2) whether the duration of effect of both components is adequate (with prolonged duration of estrogenic effect, a relative overdosage would result)

The clinical duration of effect of the mixture, therefore, can only be insufficiently compared with the effect on the vaginal epithelium because women with climactaric syndrome (hot flashes, etc.) usually spontaneously show the intermediate proliferative type, which can also be caused by androgen-estrogen mixture alone.. Very rarely do women with atrophic vaginal epithelium show deficiency symptoms. This can be shown in the study of an extensive number of cases.

When we compare the clinical efficiency (2, 3, 4, 7 weeks) with the proliferative effect on the vaginal epithelium, then we see that although wide individual range is present, the destruction of the epithelium occurs before the clinical effect decreases.

I cannot give any figures from our experience concerning the cyclic decrease in FSH due to androgens; however, it must be mentioned that in a corresponding experience with 100 mg ethinyl-19-nortestosterone enanthate, every 10 days i. m., we succeeded in inhibiting ovulation in young women and in decreasing the estrogen level to one-tenth of the normal value.

To Dr. Hochstaedt:

Dr. Hochstaedt's observations are of great practical value in addition to their theoretical significance. We found that in these women, the estrogen fraction of the androgen-estrogen had to be increased in order to eliminate the complaints. However, in spite of a ratio of estrogen-androgen 1:10, applied in these women, usually no proliferation of the vaginal epithelium appeared. The endogeneous androgen product is, therefore, an important factor and explains the different individual reactions. Whether we deal with an augmented FSH formation or a particularly good sensitivity of the androgen product in the adrenal cortex, has to be clarified by a special experimental method.

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WHAT ARE THE RELATIVE DOSAGES OF PROGESTOGENS PLUS ESTROGENS WHICH SUPPRESS THE OCCURRENCE OF THE HIGHLY PROLIFERATED CELL TYPE?

JACQUES FERIN
Louvain, Belgium

In order to resolve this problem, it is necessary to choose adequate subjects (see Topic No. 2 of this symposium), i. e., preferably surgical castrates.

A combination of estrogens and progestogens could be administered from the onset, but it seems more sure and interesting to us from a clinical viewpoint to establish artificial cycles by reproducing the sequence of the normal cycle: an estrogen treatment lasting from 14 to 16 days, sufficient to cause the appearance of an estrogenic smear, is followed by an estro-progestational treatment of equivalent duration.

Since 1940, Shorr (1) has employed a similar technique and observed that it is possible to obtain the different characteristic aspects of the normal cycle in the vaginal smears of a castrated subject by the administration of adequate dosages of estradiol benzoate and progesterone.

The recent findings of Davis, Wied and their collaborators (2, 3, 4, 5, 6) have rendered more precise information. These authors have prepared their 6 surgically castrated patients by means of an intramuscular injection of estradiol valerate or by the daily oral absorption of 1 mg. of diethylstilbestrol for 13 days.

For this determination (7) we have used two surgical castrates who had been subjected to various uninterrupted cyclic studies for many years. There had been only short intervals between the artificial cycles to allow withdrawal bleeding to occur. The proliferation phase was insured by daily oral dosages of 50 μg. of ethinyl-estradiol through 14 to 16 days.

As far as we are concerned, interpreting the question exactly, we have only considered as positive those cycles in which the highly proliferated cell type: large, flat, polygonal cells with generally eosinophilic cytoplasm and pyknotic nuclei - were only rarely (less than 1%, if at all, present. Davis and Wied did not use these critical standards in the evaluation of their smears; they speak of marked regression of proliferation, regression to middle luteal smear type or typical luteal changes.

An important preliminary finding is apparent from all of the observations: short progestational treatment (4-5 days), if it can cause a decrease of the Eosinophilic and Karyopyknotic Indices, cannot cause the disappearance of the highly proliferated cell type, no matter what the dosage employed is: 350 mg (2) or 500 mg. (6) of progesterone in a single injection; 30 mg. progesterone per day, intramuscularly (8); 25 mg. 19-norprogesterone per day, intramuscularly (7); 75 mg. 17-methyl-19-nortestosterone per day, sublingually (7); 10 mg. 17-ethinyl-19-nortestosterone per day, sublingually (7); 25 mg. 17-ethyl-19-nortestosterone per day, sublingually (7).

A prolonged progestational treatment, similar in duration to the second phase of the menstrual cycle, is necessary to obtain the disappearance of the highly proliferated cell type.

ANALYSIS OF OBSERVATIONS

The results were considered positive when the smears contained few, if any, highly proliferated cell types, and were considered negative in the inverse case.

I. Progesterone, intramuscularly:
versus 1 mg. diethylstilbestrol a day, orally:

50 or 100 mg. x 6 every other day from the 14th to the 24th day	positive (6)

II. 17-α-hydroxyprogesterone caproate, intramuscularly:

a) versus 1 mg. stilbestrol a day, orally:

150 mg., a single injection on the 14th day	negative (3)
250 mg. id.	negative (6)
350 mg. or 375 mg. id.	positive (3) (6)

b) versus 20 mg. estradiol valerate:

350 mg., a single injection on the 14th day	negative (2)

c) versus 10 mg. estradiol valerate:

350 mg., a single injection on the 14th day	positive (2)

d) versus 5 mg. estradiol valerate:

350 mg., a single injection on the 14th day	positive (2)

e) versus 250 μg. ethinyl-estradiol a day, orally:

250 mg. in 2 injections of 125 mg. on the 1st and 7th days	negative (7)

f) versus 500 μg. ethinyl-estradiol a day, orally:

250 mg. in 2 injections of 125 mg. on the 1st and 7th days	negative (7)

III. 17-α-hydroxyprogesterone acetate:

A. Intramuscularly
versus 1 mg. diethylstilbestrol a day, orally:

150 mg., a single injection on the 14th day	negative (3)
350 mg., a single injection on the 14th day	positive (3)

B. Orally
versus 1 mg. diethylstilbestrol a day, orally:

50 mg. a day	negative (4)
75 mg. a day	positive (4)

IV. 17-α-ethinyl-19-nortestosterone enanthate, intramuscularly:
versus 1 mg. diethylstilbestrol a day, orally:

100 mg., a single injection on the 14th day	negative (3)
150 mg., a single injection on the 14th day	positive (3)

V. Anhydro-hydroxyprogesterone, orally:

a) versus 1 mg. diethylstilbestrol a day, orally:

200 mg. a day	negative (4)
250 mg. a day	negative (4)
300 mg. a day	positive (6)

b) versus 100 μg. ethinyl-estradiol a day, orally:

50 mg. a day	negative (7)
200 mg. a day	negative (7)
300 mg. a day	positive (7)

c) versus 250 μg. ethinyl-estradiol a day, orally:

50 mg. a day	negative (7)
100 mg. a day	negative (7)
125 mg. a day	negative (7)
500 mg. a day	negative (7)

VI. 9-α-bromo-11-ketoprogesterone, orally:

versus 1 mg. diethylstilbestrol a day, orally:

80 mg. a day	negative (5)
100 mg. a day	positive (?) (5)

VII. 17-α-methyl-19-nortestosterone, orally:

a) versus 10 μg. ethinyl-estradiol a day, orally:

25 mg. a day	positive (7)

b) versus 25 μg. ethinyl-estradiol a day, orally:

25 mg. a day	positive (7)

c) versus 50 μg. ethinyl-estradiol a day, orally:

5 mg. a day	positive (7)
12.5 mg. a day	positive (7)

d) versus 100 μg. ethinyl-estradiol a day, orally:

5 mg. a day	positive (7)

Note that all of these smears showed a typical luteal aspect without the appearance of profound parabasal cells.

CONCLUSION

Numerous experiments will still be necessary to isolate the quantitative laws which govern the antagonism between the estrogens and progestogens on the vaginal epithelium.

We would like to underline again the extraordinary activity of the methyl derivative of 19-nortestosterone (9) (10).

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DISCUSSION

HANNS-WERNER BOSCHANN, West-Berlin, Germany:

Ferin has shown convincingly in his series of experiments that there is no generally valid estrogen:progestogen relation in which the highly proliferated cell type is suppressed. That this problem is also true for estrogen:androgen relation has been shown in the article preceding the paper by Ferin. It is always only possible in a particular case to state the behavior of a specific progestogen in relation to a specific estrogen. We have been able to obtain these results with 11 surgical castrates in continuous experiments since 1953. The following estrogens and progestogens were tested in relation to transformation dosage and effect on the vaginal epithelium.

Estrogens:		**estradiol benzoate** **estradiol valerate** **estradiol undecylate** **estradiol cyclopentylpropionate** **estrone sulfate** **ethinylestradiol** **diethylstilbestrol**
Progestogens:	**parenterally**	**chemically pure progesterone in oil** **progesterone as a crystal suspension of different grain sizes** **17-α-hydroxyprogesterone caproate** **17-α-ethinyl-nortestosterone enanthate**
	orally	**chemically pure progesterone** **anhydrohydroxyprogesterone** **17-α-methyl-19-nortestosterone** **17-α-ethyl-19-nortestosterone** **17-α-ethinyl-19-nortestosterone acetate** **17-α-hydroxyprogesterone caproate** **17-α-hydroxyprogesterone acetate** **9-α-bromo-11-ketoprogesterone**

In the inspection of our material we can completely confirm the results of Davis and Wied, of Ferin, and of Wied and co-workers in using their respective criteria. There is agreement so far as the same progestogens in same application forms and dosages are compared with the same estrogens. There would be another relation if other estrogens were used for proliferation.

This holds true for the effect on the vaginal epithelium as well as for the effect on the endometrium. Differences of various authors may, therefore, often be fictitious and may be explained by testing with various estrogens. For example, the relations of progestogens compared with estradiol benzoate should be mentioned. Five mg. of estradiol benzoate were given intramuscularly to the castrates twice weekly, all together 7 times, for a total dosage of 35 mg. Applying the criteria given by Ferin, estradiol benzoate shows the following relation to:

I.	**Progesterone, chemically pure, in oil:**	
	50 mg. singly administered on the 14th day of the artificial cycle	**Negative**
	100 mg. singly administered on the 14th day of the artificial cycle	**Negative**
	250 mg. singly administered on the 14th day of the artificial cycle	**Negative**
	500 mg. singly administered on the 14th day of the artificial cycle	**Negative**
	750 mg. singly administered on the 14th day of the artificial cycle	**Negative**
	1000 mg. singly administered on the 14th day of the artificial cycle	**Negative**
		(Rapid excretion; overflow effect!)
II.	**17-α-hydroxyprogesterone caproate i. m.**	
	65 mg. singly administered on the 14th day	**Negative**
	125 mg. singly administered on the 14th day	**Positive**
III.	**17-α-ethinyl-19-nortestosterone-enanthate i. m.**	
	50 mg. singly administered on the 14th day	**Negative**
	100 mg. singly administered on the 14th day	**Positive**
IV.	**17-α-ethinyl-19-nortestosterone, orally**	
	5 mg. daily	**Negative**
	10 mg. daily	**Positive**
V.	**17-α-ethinyl-19-nortestosterone acetate, orally**	
	3 mg. daily	**Negative**
	4 mg. daily	**Positive**

Considering the secretory changes in the endometrium after proliferation with (5x7) 35 mg. estradiol benzoate, we found the following dosages for the above mentioned progestogens:

II. 17-∝-hydroxyprogesterone caproate
(each 100 mg. on the 14th and 21st or 22nd day)

III. 17-∝-ethinyl-19-nortestosterone enanthate 100-200 mg. (50 mg.)
(once on the 14th day)

IV. 17-∝-ethinyl-19-nortestosterone 100-140 mg. (80 mg.)
(from the 14th day, daily 10 mg.)

V. 17-∝-ethinyl-19-nortestosterone acetate 30-40 mg. (20 mg.)
(from the 14th day, 3-4 mg.)

The histological picture of the late secretory phase served here as criterion (in parentheses are the threshold dosages, which caused minimal secretory changes in the endometrium).

The threshold dosage of the endometrium is therefore apparently often insufficient to induce changes in the vaginal epithelium, which Ferin uses as criteria of the suppression of estrogen proliferation. On the other hand, as Ferin's and our observations show, this dosage for the vaginal epithelium lies below the transformation dosage for the endometrium, thus between the threshold and transformation dosages.

CLOSING REMARKS

JACQUES FERIN, Louvain, Belgium:

No comments.

COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

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THE NEXT SYMPOSIUM

The Symposia by Correspondence of the next edition, Volume II, 1958, Number 3, will be devoted to the discussion of

ENDOMETRIAL CYTOLOGY

The subjects for discussion will deal with normal, functional and abnormal cytology of the endometrium as well as techniques for obtaining cytological material in this particular field of exfoliative cytology.

The following is the final list of speakers and discussants. If there is more than one contributor listed, they are listed alphabetically.

Symposium A

ENDOMETRIAL CANCER CYTOLOGY

1. Histomorphology of Endometrial Carcinoma

   JEAN de BRUX, Paris, France
   WOLFGANG KORTE, Bonn, Germany
   PETER STOLL, Heidelberg, Germany

   Disc.: Anthony F. Anderson, Edinburgh, Scotland
   Hans Bettinger, Melbourne, Australia

2. Histochemistry of Endometrial Carcinoma

   MANUEL GALBIS, Valencia, Spain
   MARGARET E. LONG, New York, New York

   Disc.: Constantin Herovici, Paris-Villejuif, France
   Walter Sandritter, Frankfurt a. M., Germany

3. Cytomorphology of Normal Endometrium

   HANNS-WERNER BOSCHANN, West-Berlin, Germany

   Disc.: Hannah Peters, Bombay, India

4. Cytochemistry of Normal and Abnormal Endometrium

   MANUEL GALBIS, Valencia, Spain

   Disc.: Pierre Haour, Lyon, France
   Margaret E. Long, New York, New York

5. Phasemicroscopy on Endometrial Cells

   HANS KLAUS ZINSER, Cologne, Germany

   Disc.: Edmund Schüller, Vienna, Austria

6. Flourescence Microscopy on Endometrial Cells

HORST SMOLKA, Kiel, Germany

Disc.: Jean Berger, Basle, Switzerland

7. Differentiation of Endocervical and Endometrial Cells

HORST SMOLKA, Kiel, Germany

Disc.: Emmerich von Haam, Columbus, Ohio
Warren R. Lang, Philadelphia, Pennsylvania
Hannah Peters, Bombay, India
Guillermo Terzano, Buenos Aires, Argentina
Luba A. Turnbull, Montreal, Canada

8. Histiocytes and Endometrial Cytology

HERBERT E. NIEBURGS, New York, New York

Disc.: Olaf T. Messelt, Oslo, Norway

9. Cytometry on Normal and Abnormal Endometrial Cells

HANNS-WERNER BOSCHANN, West Berlin, Germany
HERBERT E. NIEBURGS, New York, New York

Disc.: George L. Wied, Chicago, Illinois

10. Cytology of Endometritis

HANNS-WERNER BOSCHANN, West Berlin, Germany

Disc.: Jean de Brux, Paris, France

11. Cytology of Endometritis Tuberculosa

GUILLERMO TERZANO, Buenos Aires, Argentina

Disc.: Jorge Campos R. de C., Lima, Peru
F. A. Iklé, St. Gallen, Switzerland
Julieta C. de Laguna, Mexico, D. F., Mexico

12. Cytology of Endometrial Polyps

JEAN de BRUX and J. FROMENT-DUPRÉ, Paris, France
GUILLERMO TERZANO, Buenos Aires, Argentina

Disc.: Emmerich von Haam, Columbus, Ohio
Colette Marsan, Clichy, France
Y. S. Song, Providence, Rhode Island

13. Cytology of Endometrial Adenocarcinoma

HANNES KREMER, Vienna, Austria
GENEVIEVE DALIAN, VIOLETTE NUOVO, ARLETTE SIMATOS, Paris, France
ERICA WACHTEL, London, England

Disc.: W. Bickenbach and H. J. Soost, München, Germany
Jean de Brux, Paris, France
Manuel Galbis, Valencia, Spain
Emmerich von Haam, Columbus, Ohio
Julieta C. de Laguna, Mexico, D. F., Mexico
J. M. E. Mezzadra and G. Terzano, Buenos Aires, Argentina
George H. Romberg, White Plains, New York
Luba A. Turnbull, Montreal, Canada

14. Cytology of Endometrial Adenoacanthoma

EMMERICH von HAAM, Columbus, Ohio
LEOPOLD G. KOSS, New York, New York

Disc.: Ruth M. Graham, Buffalo, New York
F. A. Iklé, St. Gallen, Switzerland
Julieta C. de Laguna, Mexico, D. F., Mexico
Olaf T. Messelt, Oslo, Norway

15. Cytology of the Irradiated Uterine Cavity

EMMERICH von HAAM, Columbus, Ohio

Disc.: W. Bickenbach and H. J. Soost, München, Germany
Constantin Herovici, Paris-Villejuif, France

16. Cytology of Carcinoma In Situ of Endometrium

EMMERICH von HAAM, Columbus, Ohio
LEOPOLD G. KOSS, New York, New York

Disc.: Anthony F. Anderson, Edinburgh, Scotland
Jean de Brux, Paris, France
Erica Wachtel, London, England

17. Cytology of Sarcoma and Chorionepithelioma

RUTH M. GRAHAM, Buffalo, New York
VIOLETTE M. NUOVO, Paris, France
ANDRÉ RAUZY and JEAN de BRUX, Paris, France

Disc.: Hanns-Werner Boschann, West Berlin, Germany
Manuel Galbis, Valencia, Spain
F. A. Iklé, St. Gallen, Switzerland

Symposium B

TECHNIQUES FOR ENDOMETRIAL CYTOLOGY

1. Advantages and Disadvantages of Endometrial Aspiration Technique

PIERRE HAOUR, Lyon, France
B. CORNELIS HOPMAN, Miami, Florida
GUILLERMO TERZANO, Buenos Aires, Argentina

Disc.: Guiseppe Dellepiane, Torino, Italy
Manuel Galbis, Valencia, Spain
F. A. Iklé, St. Gallen, Switzerland
Colette Marsan, Clichy, Seine, France
Violette M. Nuovo, Paris, France
G. H. Romberg, White Plains, New York
Horst Smolka, Kiel, Germany

2. Advantages and Disadvantages of Endometrial Brush Technique

HANNS-WERNER BOSCHANN, West Berlin, Germany
JOSÉ MARIA E. MEZZADRA, Buenos Aires, Argentina
HERBERT E. NIEBURGS, New York, New York

Disc.: W. Bickenbach and H. J. Soost, München, Germany

3. Advantages and Disadvantages of Cervical Pessary Collection Technique

FRIEDRICH BAJARDI, Graz, Austria

Disc.: Hannah Peters, Bombay, India

4. The Diagnostic Accuracy of Vaginal Smears for Detection of Endometrial Carcinoma

RUTH M. GRAHAM, Buffalo, New York
EMMERICH von HAAM, Columbus, Ohio
ERICA WACHTEL, London, England

Disc.: Marcel Gaudefroy, Lille, France
J. M. E. Mezzadra and G. Terzano, Buenos Aires, Argentina
Violette M. Nuovo, Paris, France
Luis Montalvo-Ruiz, Madrid, Spain

5. The Diagnostic Accuracy of Cervical Smears for Detection of Endometrial Carcinoma

JULIETA CALDERON de LAGUNA, Mexico, D. F., Mexico

Disc.: A. F. Anderson, Edinburgh, Scotland
Hanns-Werner Boschann, West Berlin, Germany
Luis Montalvo-Ruiz, Madrid, Spain
Y. S. Song, Providence, Rhode Island
Guillermo Terzano, Buenos Aires, Argentina
Luba A. Turnbull, Montreal, Canada

6. Are Degenerative Cell Changes in Endometrial Cells Due to Inadequate Preparation Techniques?

J. PAUL PUNDEL, Luxembourg, Luxembourg

Disc.: Ruth M. Graham, Buffalo, New York
Pierre Haour, Lyon, France

7. Should One Routinely Perform Intra-Uterine Smears for the Detection of Endometrial Carcinoma?

HANNS-WERNER BOSCHANN, West Berlin, Germany
WARREN R. LANG, Philadelphia, Pennsylvania
EDMUND SCHÜLLER, Vienna, Austria

Disc.: W. Bickenbach and H. J. Soost, München, Germany
E. von Haam, Columbus, Ohio
F. A. Iklé, St. Gallen, Switzerland
L. A. Turnbull, Montreal, Canada

8. A Clearing Technique in Endometrial Cytology

B. CORNELIS HOPMAN, Miami, Florida

Disc.: Hanns-Werner Boschann, West Berlin, Germany
Jean de Brux, Paris, France
Guillermo Terzano, Buenos Aires, Argentina

Symposium C

ENDOMETRIAL CANCER CYTOLOGY

1. Endometrial Cytology during the Follicular Phase of the Cycle

HANNS-WERNER BOSCHANN, West Berlin, Germany
CLARICE do AMARAL FERREIRA, Rio de Janeiro, Brazil
HANNAH PETERS, Bombay, India

Disc.: W. Bickenbach and H. J. Soost, München, Germany
Manuel Galbis, Valencia, Spain
J. M. E. Mezzadra and G. Terzano, Buenos Aires, Argentina

2. Endometrial Cytology during the Luteal Phase of the Cycle

HANNS-WERNER BOSCHANN, West Berlin, Germany
CLARICE do AMARAL FERREIRA, Rio de Janeiro, Brazil
HANNAH PETERS, Bombay, India

Disc.: W. Bickenbach and H. J. Soost, München, Germany
Manuel Galbis, Valencia, Spain

3. Endometrial Cytology during and after the Menopause

HANNS-WERNER BOSCHANN, West Berlin, Germany

Disc.: W. Bickenbach and H. J. Soost, München, Germany
Jean de Brux, Paris, France
George H. Romberg, White Plains, New York

4. Endometrial Cytology in Endometrial Hyperplasia

ARTURO ANGEL ARRIGHI, Buenos Aires, Argentina
JEAN de BRUX, Paris, France
CLARICE do AMARAL FERREIRA, Rio de Janeiro, Brazil

Disc.: W. Bickenbach and H. J. Soost, München, Germany
Hanns-Werner Boschann, West Berlin, Germany
Marcel Gaudefroy, Lille, France
Ruth M. Graham, Buffalo, New York
Julieta C. de Laguna, Mexico, D.F., Mexico
Y. S. Song, Providence, Rhode Island
G. Terzano and J. M. E. Mezzadra, Buenos Aires, Argentina

5. Diagnostic Accuracy of Hormonal Evaluation by Means of Endometrial Cytology

GEORGE H. ROMBERG, White Plains, New York

Disc.: Hanns-Werner Boschann, West Berlin, Germany
Clarice do Amaral Ferreira, Rio de Janeiro, Brazil

6. Correlative Studies of Endometrial Cytology, Endometrial Histology and Vaginal Cytology

WINIFRED LIU and RUTH M. GRAHAM, Buffalo, New York
LUIS MONTALVO-RUIZ, Madrid, Spain
PIERO SORA, Pavia, Italy

Disc.: Jean de Brux, Paris, France
Marcel Gaudefroy, Lille, France
Wolfgang Korte, Bonn, Germany

7. Hormonal Evaluation by Means of Vaginal Cytology of Patients with Endometrial Carcinoma

GEORGE L. WIED, Chciago, Illinois

Disc.: W. Bickenbach and H. J. Soost, München, Germany
Hanns-Werner Boschann, West Berlin, Germany
Ruth M. Graham, Buffalo, New York
Pierre Haour, Lyon, France
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FUTURE SYMPOSIA

VOLUME III 1959 NUMBER 1

The Symposia by Correspondence of this edition will be devoted to the discussion of:

CYTOLOGY DURING PREGNANCY

The subjects for discussion will cover normal and disturbed pregnancy and cervical carcinoma during pregnancy.

Speakers are listed alphabetically.

The following is the final program and list of speakers and discussants (December 1, 1958).

Symposium A

CANCER CYTOLOGY IN PREGNANCY,

1. The Squamous Columnar Junctions during Pregnancy

   KARL-GÜNTHER OBER, Cologne, Germany

   Disc.: Alvan G. Foraker, Jacksonville, Florida, U. S. A.
   Dean L. Moyer and Louis J. Zeldis, Los Angeles, California, U. S. A.

2. Incidence of Cervical Carcinoma during Pregnancy

   JORGE CAMPOS R. de C., Lima, Peru
   JOHN B. GRAHAM, Buffalo, New York, U. S. A.
   JULIETA C. de LAGUNA and MARIA I. ALGORRI, Mexico, D. F., Mexico
   THOMAS A. SLATE, San Diego, California, U. S. A.
   PETER STOLL, Heidelberg, Germany

   Disc.: George J. Andros, Philadelphia, Pennsylvania, U. S. A.
   Ronald R. Greene, Chicago, Illinois, U. S. A.
   Emmerich von Haam, Columbus, Ohio, U. S. A.
   Edmund Schüller, Vienna, Austria

3. Prognosis of Cervical Carcinoma during Pregnancy as Compared with the Prognosis of Cervical Carcinoma in Non-Pregnant Women

   W. BICKENBACH and H. J. SOOST, Munich, Germany
   G. DALIAN, A. SIMATOS and V. NUOVO, Paris, France
   HERBERT E. NIEBURGS, New York, New York, U. S. A.
   EDMUND SCHÜLLER, Vienna, Austria

   Disc.: Alfred Glücksmann, Cambridge, England, U. K.
   Jules-André Bret, Paris, France
   Luis Montalvo-Ruiz, Madrid, Spain
   Peter Stoll, Heidelberg, Germany

4. Histomorphology of Cervical Carcinoma during Pregnancy

FRIEDRICH BAJARDI, Graz, Austria
JEAN de BRUX, Paris, France
JORGE CAMPOS R. de C., Lima, Peru
ROGER VOKAER, Brussels, Belgium

Disc.: Alvan G. Foraker, Jacksonville, Florida, U.S.A.
Alfred Glücksmann, Cambridge, England, U.K.
Emmerich von Haam, Columbus, Ohio, U.S.A.
Dean L. Moyer and Louis J. Zeldis, Los Angeles, California, U.S.A.
Herbert E. Nieburgs, New York, New York, U.S.A.

5. Histomorphology of Cervical Decidual Reaction

JEAN de BRUX and JACQUELINE DUPRÉ-FROMENT, Paris, France

6. Colposcopy during Pregnancy and the Post-Partum Period (on Normal and Abnormal Cervices)

ALFONSO ALVAREZ-BRAVO, Mexico, D. F., Mexico
JULES-ANDRÉ BRET and F. J. COUPEZ, Paris, France
WARREN R. LANG, Philadelphia, Pennsylvania, U.S.A.

Disc.: Friedrich Bajardi, Graz, Austria
Jean Berger, Basel, Switzerland
Raimund Krimmenau, Dresden, Germany
Jacques W. Jenny, Alfred Wacek, Hans Iselin Wyes and Ulrich Baumann, Zürich, Switzerland
Otakar Nykliček, Náchod, Czechoslovakia

7. Colpomicroscopy during Pregnancy (on Normal and Abnormal Epithelial Areas)

T. ANTOINE, K. BRANDL, V. GRÜNBERGER, E. KOFLER and H. KREMER, Vienna, Austria
WOLFGANG WALZ, Heidenheim a.d., Brenz, Germany

Disc.: John F. Sheehan, Omaha, Nebraska, U.S.A.

8. Exfoliative Cytology of Infections during Pregnancy

EMMERICH von HAAM, Columbus, Ohio, U.S.A.
HERBERT E. NIEBURGS, New York, New York, U.S.A.

Disc.: Luis Montalvo-Ruiz, Madrid, Spain

9. Exfoliative Cytology of Dysplasia during Pregnancy

G. DALIAN, A. SIMATOS and VIOLETTE NUOVO, Paris, France
EMMERICH von HAAM, Columbus, Ohio, U.S.A.
ROBERT E. L. NESBITT, Jr., DORIS S. ROME and ARTHUR A. STEIN, Albany, New York, U.S.A.
GUILLERMO TERZANO, Buenos Aires, Argentina

Disc.: George J. Andros, Philadelphia, Pennsylvania, U.S.A.
Jean Berger, Basel, Switzerland
Jean de Brux, Paris, France
Camille Lichtfus, Athus, Belgium
José Maria E. Mezzadra, Buenos Aires, Argentina
Dean L. Moyer and Louis J. Zeldis, Los Angeles, California, U.S.A.

10. Exfoliative Cytology of Carcinoma in Situ during Pregnancy

JEAN de BRUX, Paris, France
G. DALIAN, A. SIMATOS and VIOLETTE NUOVO, Paris, France
JULIETA C. de LAGUNA, Mexico, D.F., Mexico
HERBERT E. NIEBURGS, New York, New York, U.S.A.

Disc.: Anthony F. Anderson, Edinburgh, Scotland, U. K.
George J. Andros, Philadelphia, Pennsylvania, U. S. A.
J. Ernest Ayre, Miami, Florida, U. S. A.
Jean Berger, Basel, Switzerland
Hanns-Werner Boschann, West-Berlin, Germany
Jacques W. Jenny, Alfred Wacek, Hans Iselin Wyss and Ulrich Baumann, Zurich, Switzerland
Luis Montalvo-Ruiz, Madrid, Spain

11. Exfoliative Cytology of Invasive Carcinoma during Pregnancy

EMMERICH von HAAM, Columbus, Ohio, U. S. A.

Disc.: Jean Berger, Basel, Switzerland
Hanns-Werner Boschann, West-Berlin, Germany
Jorge Campos, R. de C., Lima, Peru
F. A. Iklé, St. Gallen, Switzerland

12. Clinical Viewpoints with Special Consideration to Therapy and Time of Therapy for Lesions of the Uterine Cervix during Pregnancy

W. BICKENBACH and H. J. SOOST, Munich, Germany
JULES-ANDRÉ BRET and F. J. COUPEZ, Paris, France
JULIETA C. de LAGUNA, Mexico, D. F., Mexico

Disc.: George J. Andros, Philadelphia, Pennsylvania, U. S. A.
Jean Berger, Basel, Switzerland
Hanns-Werner Boschann, West-Berlin, Germany
Ronald R. Greene, Chicago, Illinois, U. S. A.
Edmund Schüller, Vienna, Austria
Peter Stoll, Heidelberg, Germany

13. Diagnostic Accuracy of Colposcopy as Compared to Cytology in the Detection of Cervical Carcinoma during Pregnancy

FRIEDRICH BAJARDI, Graz, Austria
JULES-ANDRÉ BRET and F. J. COUPEZ, Paris, France
JEAN de BRUX, Paris, France

Disc.: Jean Berger, Basel, Switzerland
Alfonso Alvarez-Bravo, Mexico, D. F., Mexico
Clarice do Amaral Ferreira, Rio de Janeiro, Brazil
F. A. Iklé, St. Gallen, Switzerland
Jacques W. Jenny, Alfred Wacek, Hans Iselin Wyss and Ulrich Baumann, Zürich, Switzerland
Warren R. Lang, Philadelphia, Pennsylvania, U. S. A.

14. Should All Pregnant Women be Screened for Cervical Carcinoma?

EMMERICH von HAAM, Columbus, Ohio, U. S. A.
LEOPOLD G. KOSS, New York, New York, U. S. A.
THOMAS A. SLATE, San Diego, California, U. S. A.

Disc.: George J. Andros, Philadelphia, Pennsylvania, U. S. A.
J. Ernest Ayre, Miami, Florida, U. S. A.
Hanns-Werner Boschann, West-Berlin, Germany
Jules-André Bret, Paris, France
Ronald R. Greene, Chicago, Illinois, U. S. A.
Julieta C. de Laguna, Mexico, D. F., Mexico
Jacques W. Jenny, Alfred Wacek, Hans Iselin Wyss and Ulrich Baumann, Zurich, Switzerland
Warren R. Lang, Philadelphia, Pennsylvania, U. S. A.
Violette M. Nuovo, Paris, France
Y. S. Song, Providence, Rhode Island, U. S. A.

15. Exfoliative Cytology and Colposcopy of Cervical Decidual Reaction

JULES-ANDRÉ BRET and F. J. COUPEZ, Paris, France
ELIE TASSO, ANDRÉ RAUZY and JEAN de BRUX, Paris, France

Disc.: Violette M. Nuovo, Paris, France

Symposium B

HORMONAL CYTOLOGY DURING PREGNANCY AND POST-PARTUM PERIOD

1. The Rate of Production of Endogenous Hormones in Pregnancy

   JOSEF ZANDER, Cologne, Germany

2. Hormonal Basis of Cytological Changes During Pathological Pregnancy

   OTTO STAMM and GUSTAVE RIOTTON, Geneva, Switzerland

3. Epithelial and Connective Tissue during Pregnancy

   JEAN de BRUX, Paris, France

4. Normal Vaginal Cytology during Pregnancy

   EMMERICH von HAAM and T. D. EFSTATION, Columbus, Ohio, U. S. A.
   BERTHOLD B. HOCHSTAEDT, Haifa, Israel
   HERBERT E. NIEBURGS, New York, New York, U. S. A.
   J. PAUL PUNDEL, Luxembourg, Luxembourg

   Disc.: Marcel Gaudefroy, Lille, France
   Constantin Herovici, Villejuif, Seine, France
   Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
   Raimund Krimmenau, Dresden, Germany
   José Maria E. Mezzadra, Buenos Aires, Argentina
   H. Muth, Münster, Westfalen, Germany
   Guillermo Terzano, Buenos Aires, Argentina

5. Incidence of Cytolysis in Smears during Pregnancy

   MARCEL GAUDEFROY, Lille, France
   EMMERICH von HAAM and ROBERT SCHWALLENBERG, Columbus, Ohio, U. S. A.
   J. PAUL PUNDEL, Luxembourg, Luxembourg

   Disc.: Jacques Ferin, Louvain, Belgium
   Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
   Camille Lichtfus, Athus, Belgium
   Herbert E. Nieburgs, New York, New York, U. S. A.

6. Vaginal Flora of Pregnant Women as Compared to That of Non-Pregnant Women

   ARTURO ANGEL ARRIGHI, Buenos Aires, Argentina
   JULES-ANDRÉ BRET, Paris, France
   OTAKAR NYKLÍČEK, Náchod, Czechoslovakia

   Disc.: Jacques Ferin, Louvain, Belgium
   Emmerich von Haam, Columbus, Ohio, U. S. A.
   Berthold B. Hochstaedt, Haifa, Israel
   Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
   J. Paul Pundel, Luxembourg, Luxembourg

7. Vaginal Cytology as a Prognostic Method in Pregnancy Disorders

   MARCEL GAUDEFROY, Lille, France
   LUIS MONTALVO-RUIZ, Madrid, Spain
   J. PAUL PUNDEL, Luxembourg, Luxembourg

   Disc.: Jules-André Bret, Paris, France
   Jacques Ferin, Louvain, Belgium
   Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
   José Maria E. Mezzadra, Buenos Aires, Argentina
   Herbert E. Nieburgs, New York, New York, U. S. A.
   Erica Wachtel, London, England, U. K.

8. Effect of Administered Estrogens on the Vaginal Epithelium in Pregnancy and Post-Partum

MARIO de BENNING KAMNITZER, Rio de Janeiro, Brazil
J. PAUL PUNDEL, Luxembourg, Luxembourg

Disc.: Jules-André Bret, Paris, France
Jacques Ferin, Louvain, Belgium
Marcel Gaudefroy, Lille, France
Julieta C. de Laguna, Mexico, D. F., Mexico
František Horalek and Mojmír Sonek, Brno, Czechoslovakia
Luis Montalvo-Ruiz, Madrid, Spain

9. Vaginal Cytology at the End of Pregnancy

CAMILLE LICHTFUS, Strasbourg, France
NILO P. LUZ, Porto Alegre, Brazil
LUIS MONTALVO-RUIZ, Madrid, Spain
J. PAUL PUNDEL, Luxembourg, Luxembourg

Disc.: František Horalek and Mojmír Sonek, Brno, Czechosolvakia
Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
José Maria E. Mezzadra, Buenos Aires, Argentina
Otakar Nyklíček, Náchod, Czechoslovakia
Gustave Riotton, Geneva, Switzerland
Guillermo Terzano, Buenos Aires, Argentina
Julieta C. de Laguna, Mexico, D. F., Mexico

10. Vaginal Cytology after Rupture of Fetal Membranes

B. CORNELIS HOPMAN, Miami, Florida, U. S. A.

Disc.: Emmerich von Haam, Columbus, Ohio, U. S. A.
František Horalek and M. Sonek, Brno, Czechoslovakia
Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
Violette M. Nuovo, Paris, France
J. Paul Pundel, Luxembourg, Luxembourg

11. Vaginal Cytology Post-Partum and During Lactation Period

MARIO de BENNING KAMNITZER, Rio de Janeiro, Brazil
WARREN R. LANG, Philadelphia, Pennsylvania, U. S. A.
OTAKAR NYKLÍČEK, Náchod, Czechoslovakia
HANNAH PETERS, Bombay, India
ROGER VOKAER, Brussels, Belgium

Disc.: J. Ernest Ayre, Miami, Florida, U. S. A.
František Horalek and Mojmír Sonek, Brno, Czechoslovakia
Julieta C. de Laguna, Mexico, D. F., Mexico
José Maria E. Mezzadra, Buenos Aires, Argentina
Violette M. Nuovo, Paris, France
J. Paul Pundel, Luxembourg, Luxembourg
Y. S. Song, Providence, Rhode Island, U. S. A.
Peter Stoll, Heidelberg, Germany
Guillermo Terzano, Buenos Aires, Argentina

12. Vaginal Cytology in Abortion

GIUSEPPE DELLEPIANE, Torino, Italy
CLARICE do AMARAL FERREIRA, Rio de Janeiro, Brazil
OTTO STAMM, V. RAWYLER and GUSTAVE RIOTTON, Geneva, Switzerland

Disc.: George J. Andros, Philadelphia, Pennsylvania, U. S. A.
Jean de Brux, Paris, France
Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
José Maria E. Mezzadra, Buenos Aires, Argentina
Herbert E. Nieburgs, New York, New York, U. S. A.
Gustave Riotton, Geneva, Switzerland
Guillermo Terzano, Buenos Aires, Argentina

13. Vaginal Cytology in Ectopic Pregnancy

ARTURO ANGEL ARRIGHI, Buenos Aires, Argentina

Disc.: J. Ernest Ayre, Miami, Florida, U.S.A.
W. Bickenbach and H. J. Soost, Munich, Germany
Jean de Brux, Paris, France
Hanns-Werner Boschann, West-Berlin, Germany

14. Diagnosis of Pregnancy by Means of Cytology

B. CORNELIS HOPMAN, Miami, Florida, U.S.A.
J. PAUL PUNDEL, Luxembourg, Luxembourg

Disc.: Marcel Gaudefroy, Lille, France
František Horalek and M. Sonek, Brno, Czechoslovakia
Mario de Benning Kamnitzer, Rio de Janeiro, Brazil
Julieta C. de Laguna, Mexico, D.F., Mexico
Hannah Peters, Bombay, India

15. Urinary Cytology During Pregnancy

MARIO GONZALES RAMOS, Mexico, D.F., Mexico
GUILLERMO TERZANO and ARTURO A. ARRIGHI, Buenos Aires, Argentina

16. Correlative Studies on Vaginal, Urethral, Oral Smears and Smears from the Urinary Sediment During Normal Pregnancy

PIERO SORA, Pavia, Italy

Disc.: W. Bickenbach and H. J. Soost, Munich, Germany

VOLUME III 1959 NUMBER 2

The symposia by correspondence of this edition will be devoted to the discussion of:

R A D I A T I O N C E L L C H A N G E S

Deadlines for Contributions to the Symposia of this Edition:

FOR BEING LISTED AS SPEAKER OR DISCUSSANT:

In order to be listed as Main Speaker or Discussant on any of the topics listed in the following program, participants should inform the Editorial Office as soon as possible; however, NOT LATER THAN: February 15, 1959, about their intention to contribute.

FOR RECEIPT OF MAIN PAPERS

Main papers in the English language must REACH ACTA CYTOLOGICA, Editorial Office, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A., NOT LATER THAN: March 1, 1959.

Main papers in French, German, or Spanish must REACH ACTA CYTOLOGICA, Editorial Office NOT LATER THAN: February 10, 1959, to permit translation and subsequent approval by author.

FOR RECEIPT OF DISCUSSION:

THE DISCUSSANTS WILL RECEIVE THE PAPERS OF THE MAIN SPEAKERS FOR COMMENTS AS SOON AS THEY ARE AVAILABLE.

Discussions in the English language must REACH ACTA CYTOLOGICA, Editorial Office, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A., NOT LATER THAN: May 15, 1959.

Discussions in French, German or Spanish must REACH ACTA CYTOLOGICA, Editorial Office NOT LATER THAN: April 25, 1959, to permit translation and subsequent approval by author.

FOR RECEIPT OF CLOSING REMARKS BY MAIN SPEAKERS:

THE MAIN SPEAKERS WILL RECEIVE THE DISCUSSIONS FOR CLOSING REMARKS AS SOON AS THEY ARE AVAILABLE.

Closing remarks in the English language must REACH the Editorial Office NOT LATER THAN: August 1, 1959. Closing remarks in French, German or Spanish must REACH the Editorial Office NOT LATER THAN: July 10, 1959.

PRELIMINARY PROGRAM OF VOLUME III, NUMBER 2, 1959

DEFINITION, MORPHOLOGY, CYTOCHEMISTRY, DIAGNOSTIC AND PROGNOSTIC IMPORTANCE OF RADIATION CHANGES OF BENIGN (RR-) CELLS AND OF TUMOR CELLS IN THE FEMALE GENITAL TRACT

1. Definition

RUTH M. GRAHAM, Buffalo, New York, U. S. A.
Disc.: Olle Kjellgren, Gothenburg, Sweden

2. Morphology

ARTURO ANGEL ARRIGHI, Buenos Aires, Argentina
RUTH M. GRAHAM, Buffalo, New York, U. S. A.
OLLE KJELLGREN, Gothenburg, Sweden
HORST SMOLKA, Kiel, Sweden
ERICA WACHTEL, London, England, U. K.
Disc.: Emmerich von Haam, Columbus, Ohio, U. S. A.
Ludwig von Bertalanffy, Topeka, Kansas, U. S. A.

3. Cytochemistry of Irradiated Cells

CONSTANTIN HEROVICI, Villejuif, Seine, France
PIERRE HAOUR, Lyon, France
Disc.: Ludwig von Bertalanffy, Topeka, Kansas, U. S. A.

4. Phasemicroscopy

EDMUND SCHÜLLER, Vienna, Austria

5. UV-Microscopy

6. Fluoresence Microscopy

EMMERICH von HAAM, Columbus, Ohio, U. S. A.
Disc.: Ludwig von Bertalanffy, Topeka, Kansas, U. S. A.

8. Colpomicroscopy of Irradiated Cervix

WOLFGANG WALZ, Heidenheim a. d., Brenz, Germany

9. Colposcopy of Irradiated Cervix

FRIEDRICH BAJARDI, Graz, Austria
WARREN R. LANG, Philadelphia, Pennsylvania, U. S. A.
Disc.: Otakar Nyklíček, Náchod, Czechoslovakia

10. Tissue Culture

11. Animal Experiments

EMMERICH von HAAM, Columbus, Ohio, U. S. A.

Disc.: Ludwig von Bertalanffy, Topeka, Kansas, U. S. A.

12. Minimal r-Dosages of X-ray or Minimal mg-E-h Dosages of Radium Which Produce Radiation Cell Changes

EDMUND SCHÜLLER, Vienna, Austria

Disc.: Olle Kjellgren, Gothenburg, Sweden

13. Histological Criteria of Radiation Response

ALFRED GLÜCKSMANN, Cambridge, England, U. K.

Disc.: Emmerich von Haam, Columbus, Ohio, U. S. A.
Ludwig von Bertalanffy, Topeka, Kansas, U. S. A.
Antoine Zajdela and Michel Coton, Paris, France

14. Prognosis by Means of Histology (Method)

(a) Prognosis based on tumor type and on presence of lymphatic emboli

ALFRED GLÜCKSMANN, Cambridge, England, U. K.
COLETTE MARSAN, Clichy, Seine, France

(b) Prognosis based on serial biopsies

ALFRED GLÜCKSMANN, Cambridge, England, U. K.
COLETTE MARSAN, Clichy, Seine, France

Disc.: Constantin Herovici, Villejuif, Seine, France
Jorge Campos R. de C., Lima, Peru
Claud W. Taylor, Birmingham, England, U. K.

15. Do Serial Biopsies Disturb the Healing Process of the Irradiated Cervical Carcinoma?

Disc.: Alfred Glücksmann, Cambridge, England, U. K.

16. Prognosis by Means of Exfoliative Cytology (Method)

RUTH M. GRAHAM, Buffalo, New York, U. S. A.
OLLE KJELLGREN, Gothenburg, Sweden
JULIETA C. de LAGUNA, Mexico, D. F., Mexico

Disc.: Emmerich von Haam, Columbus, Ohio, U. S. A.
F. A. Iklé, St. Gallen, Switzerland
Horst Smolka, Kiel, Germany

17. Comparative Studies of Exfoliative Cytology and Histology After Irradiation

JORGE CAMPOS R. de C., Lima, Peru
CONSTANTIN HEROVICI, Villejuif, Seine, France
DAVID WOOD and JAMES MERRILL, San Francisco, California, U. S. A.

18. Does Irradiation Influence the Karyopyknotic Index?

OLLE KJELLGREN, Gothenburg, Sweden
GUILLERMO TERZANO, Buenos Aires, Argentina

Disc.: Julieta C. de Laguna, Mexico, D. F., Mexico
Otakar Nyklíček, Náchod, Czechoslovakia

19. Recurrent Carcinoma and Presence of Radiation Cell Changes

ARTURO ANGEL ARRIGHI, Buenos Aires, Argentina
EMMERICH von HAAM, Columbus, Ohio, U. S. A.

20. Is There a Difference in Cellular Response (Concerning Onset of Response, Quality and Quantity) if Irradiation Has Been Only by X-Ray, Only by Radium, or Both? If so, What are the Differences?

Disc.: Constantin Herovici, Villejuif, Seine, France
Pierre Haour, Lyon, France
Julieta C. de Laguna, Mexico, D. F., Mexico

21. Are the Degree and the Duration of Radiation Cell Changes Dependent on the Dosage of Irradiation or the Period of Time Over Which it is Given?

OLLE KJELLGREN, Gothenburg, Sweden

Disc.: Jorge Campos R. de C., Lima, Peru
J. Edward Hall, Brooklyn, New York, U. S. A.
Pierre Haour, Lyon, France
Julieta C. de Laguna, Mexico, D. F., Mexico

22. Can the Cytological or Histological Response to Irradiation be Influenced by Medication or Other Factors?

OLLE KJELLGREN, Gothenburg, Sweden
B. CORNELIS HOPMAN, Miami, Florida, U. S. A.

Disc.: Warren R. Lang, Philadelphia, Pennsylvania, U. S. A.
Julieta C. de Laguna, Mexico, D. F., Mexico
J. Paul Pundel, Luxembourg, Luxembourg

23. Clinical Viewpoint: Should a Lesion be Treated with Surgery After it Has Been Shown Cytologically That There is Not Any - or Only Slight - Radiation Response Present? If so, When Should Surgery be Performed?

JOHN B. GRAHAM, Buffalo, New York, U. S. A.
COLETTE MARSAN, Clichy, Seine, France

Disc.: J. Edward Hall, Brooklyn, New York, U. S. A.
F. A. Iklé, St. Gallen, Switzerland
Olle Kjellgren, Gothenburg, Sweden
Julieta C. de Laguna, Mexico, D. F., Mexico

24. Clinical Viewpoint: Radical Surgery in Cases of Recurrent Cervical Carcinoma After Irradiation

JUAN CARLOS AHUMADA, Buenos Aires, Argentina
JOHN B. GRAHAM, Buffalo, New York, U. S. A.
JOE VINCENT MEIGS, Boston, Massachusetts, U. S. A.

Disc.: F. A. Iklé, St. Gallen, Switzerland
Olle Kjellgren, Gothenburg, Sweden

25. Which Clinical Factors are Associated with a Good Cytological Response?

OLLE KJELLGREN, Gothenburg, Sweden

Disc.: Jorge Campos R. de C., Lima, Peru
Julieta C. de Laguna, Mexico, D. F., Mexico

26. Cytological Response as Compared with Local Primary Healing and with Local Recurrence and Metastases of Cervical Carcinoma

OLLE KJELLGREN, Gothenburg, Sweden

Disc.: F. A. Iklé, St. Gallen, Switzerland
Julieta C. de Laguna, Mexico, D. F., Mexico

27. Comparison of Radiation Cell Changes in Exfoliated Vaginal Cells and Exfoliated Cells Elsewhere, e. g., Oral Carcinoma

MARCEL DARGENT and JACQUELINE MOURIQUAND, Lyon, France
PIERRE HAOUR, Lyon, France
HANNAH PETERS, Bombay, India

28. How Soon After the Beginning of Therapy Can the Radiation Response be Judged Accurately?

OLLE KJELLGREN, Gothenburg, Sweden

Disc.: Alfred Glücksmann, Cambridge, England, U.K.
Emmerich von Haam, Columbus, Ohio, U.S.A.
Pierre Haour, Lyon, France
Julieta C. de Laguna, Mexico, D.F., Mexico
Horst Smolka, Kiel, Germany

29. Results of RR-Cell Studies (Minimal Requirements for Participants in this Topic: 100 Patients Studied Over a Period of 5 Years)

RUTH M. GRAHAM, Buffalo, New York, U.S.A.
OLLE KJELLGREN, Gothenburg, Sweden

Disc.: Julieta C. de Laguna, Mexico, D.F., Mexico

PLEASE NOTE: THE SECOND SYMPOSIUM PLANNED FOR THIS EDITION ENTITLED
SYMPOSIUM ON SR-CELLS
WILL BE HELD AT A LATER DATE.

VOLUME III 1959 NUMBER 3

The symposia by correspondence of this edition will be devoted to the discussion of two main subjects:

A. EFFECTS OF ENDOGENOUS ESTROGENS ON THE VAGINAL EPITHELIUM
B. VARIOUS TECHNIQUES OF OBTAINING MATERIAL FOR CYTOLOGICAL STUDIES

Deadlines for Contributors to the Symposia of this Edition:

FOR BEING LISTED AS SPEAKER OR DISCUSSANT:

In order to be listed as a Main Speaker or Discussant on any of the topics listed in the following program, participants should inform the Editorial Office as soon as possible; however, NOT LATER THAN: May 1, 1959, about their intention to contribute.

FOR RECEIPT OF MAIN PAPERS:

Main papers in the English language must REACH ACTA CYTOLOGICA, Editorial Office, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A., NOT LATER THAN: July 1, 1959.

Main papers in French, German or Spanish must REACH ACTA CYTOLOGICA, Editorial Office NOT LATER THAN: June 10, 1959, to permit translation and subsequent approval by author.

FOR RECEIPT OF DISCUSSIONS:

THE DISCUSSANTS WILL RECEIVE THE PAPERS OF THE MAIN SPEAKERS FOR COMMENTS AS SOON AS THEY ARE AVAILABLE.

Discussions in the English language must REACH ACTA CYTOLOGICA, Editorial Office, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A., NOT LATER THAN: September 15, 1959.

Discussions in French, German or Spanish must REACH ACTA CYTOLOGICA, Editorial Office NOT LATER THAN: August 20, 1959, to permit translation and subsequent approval by author.

FOR RECEIPT OF CLOSING REMARKS BY MAIN SPEAKERS:

THE MAIN SPEAKERS WILL RECEIVE THE DISCUSSIONS FOR CLOSING REMARKS AS SOON AS THEY ARE AVAILABLE.

Closing remarks in the English language must REACH the Editorial Office NOT LATER THAN: December 1, 1959. Closing remarks in French, German or Spanish must REACH the Editorial Office NOT LATER THAN: November 10, 1959.

PRELIMINARY PROGRAM OF
VOLUME III, NUMBER 3, 1959

Symposium A

EFFECTS OF ENDOGENOUS ESTROGENS ON THE VAGINAL EPITHELIUM

1. Introduction. Sources of Endogenous Estrogens

2. Histological Changes in the Epithelium and Connective Tissue of the Vagina and Ectocervix as a Result of Physiological Presence, Deficiency or Absence of Endogenous Estrogen Stimulation

   JEAN de BRUX, Paris, France

3. Cytological Changes of Vaginal and Ectocervix Epithelium as a Result of Physiological Presence, Deficiency or Absence of Endogenous Estrogen Stimulation

   EMMERICH von HAAM, Columbus, Ohio, U. S. A.
   HANNAH PETERS, Bombay, India

4. Cytological Changes of the Endocervical Epithelium as a Result of Physiological Presence, Deficiency or Absence of Endogenous Estrogen Stimulation

   JEAN de BRUX, Paris, France
   HORST SMOLKA, Kiel, Germany

5. Cytochemistry of Exfoliated Atrophic Cells (Basal-Parabasal Cell)

   CONSTANTIN HEROVICI, Villejuif, Seine, France
   HERBERT E. NIEBURGS, New York, New York, U. S. A.
   Disc.: Jean de Brux, Paris, France

6. Cytochemistry of Exfoliated Intermediate Cells

   CONSTANTIN HEROVICI, Villejuif, Seine, France
   HERBERT E. NIEBURGS, New York, New York, U. S. A.
   Disc.: Pierre Haour, Lyon, France

7. Cytochemistry of Exfoliated Superficial Cells and Anucleated Squamous Cells

   CONSTANTIN HEROVICI, Villejuif, Seine, France
   HERBERT E. NIEBURGS, New York, New York, U. S. A.

8. Cytochemistry of Cytoplasmic Granules

   CONSTANTIN HEROVICI, Villejuif, Seine, France

9. Terminology of Cytological Smears in Regard to Estrogen Effect

   EMMERICH von HAAM, Columbus, Ohio, U. S. A.
   HERBERT E. NIEBURGS, New York, New York, U. S. A.
   ABRAHAM E. RAKOFF, Philadelphia, Pennsylvania, U. S. A.

Disc.: Marcel Gaudefroy, Lille, France
J. Paul Pundel, Luxembourg, Luxembourg

10. Is the Karyopyknotic Index a Measurement of Endogenous Estrogen?

HERBERT E. NIEBURGS, New York, New York, U. S. A.
GUILLERMO TERZANO, Buenos Aires, Argentina
ERICA WACHTEL, London, England, U. K.

Disc.: J. Paul Pundel, Luxembourg, Luxembourg
Marcel Gaudefroy, Lille, France

11. Is the Presence of Epithelial Atrophy a Definite Criteria of Lack of Estrogen Production?

ARTURO ANGEL ARRIGHI, Buenos Aires, Argentina
JEAN de BRUX, Paris, France
ERICA WACHTEL, London, England, U. K.

Disc.: Marcel Gaudefroy, Lille, France
Pierre Haour, Lyon, France
Otakar Nyklíček, Náchod, Czechoslovakia
J. Paul Pundel, Luxembourg, Luxembourg

12. Cellular Degeneration (Cytolysis, Autolysis) and Endogenous Estrogens

Disc.: Pierre Haour, Lyon, France
Horst Smolka, Kiel, Germany

13. The Value of Exfoliative Cytology in the Diagnosis of Ovulation

MARCEL GAUDEFROY, Lille, France
ABRAHAM E. RAKOFF, Philadelphia, Pennsylvania, U. S. A.
ERICA WACHTEL, London, England, U. K.

Disc.: Jean de Brux, Paris, France
Pierre Haour, Lyon, France
J. Paul Pundel, Luxembourg, Luxembourg
Guillermo Terzano, Buenos Aires, Argentina

14. The Value of Exfoliative Cytology in the Diagnosis of Follicular Persistency

JEAN de BRUX, Paris, France
HERBERT E. NIEBURGS, New York, New York, U. S. A.

Disc.: Arturo Angel Arrighi, Buenos Aires, Argentina
Otakar Nyklíček, Náchod, Czechoslovakia
J. Paul Pundel, Luxembourg, Luxembourg

15. Vaginal Cytology in Hysterectomized Patients, with Special Reference to Presence or Absence of Ovarian Function After Hysterectomy

PIERRE HAOUR, Lyon, France
ERICA WACHTEL, London, England, U. K.
GEORGE L. WIED, Chicago, Illinois, U. S. A.

Disc.: Emmerich von Haam, Columbus, Ohio, U. S. A.
H. Muth, Muenster, Westfalen, Germany
J. Paul Pundel, Luxembourg, Luxembourg

16. The Value of Exfoliative Cytology in the Diagnosis of Hormone-Producing Tumors

JEAN de BRUX, Paris, France
MARCEL GAUDEFROY, Lille, France
EDMUND SCHÜLLER, Vienna, Austria

Disc.: Abraham E. Rakoff, Philadelphia, Pennsylvania, U. S. A.
Erica Wachtel, London, England, U. K.

17. The Level of Endogenous Estrogens in Patients with Cervical Carcinoma as Demonstrated by Methods Other Than Exfoliative Cytology

18. The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Cervical Carcinoma

HERBERT E. NIEBURGS, New York, New York, U.S.A.

Disc.: Emmerich von Haam, Columbus, Ohio, U.S.A.

19. The Level of Endogenous Estrogens in Patients with Ovarian or Endometrial Carcinoma as Demonstrated by Methods Other Than Exfoliative Cytology

ABRAHAM E. RAKOFF, Philadelphia, Pennsylvania

20. The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Ovarian or Endometrial Carcinoma

HERBERT E. NIEBURGS, New York, New York, U.S.A.

21. The Level of Endogenous Estrogens in Patients with Breast Carcinoma as Demonstrated by Methods Other Than Exfoliative Cytology

22. The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Untreated, Treated and Recurrent Breast Carcinoma (Excluding Those Cases Receiving Hormone Therapy or Who Have Undergone Extirpation of Endocrine Organs)

JOHN A. FINKBEINER, New York, New York, U.S.A.

Disc.: Pierre Haour, Lyon, France

23. The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Breast Carcinoma after Ovariectomy (Those Treated by Sex Steroids and Those Without Additional Sex Steroids Administration Considered Separately)

JOHN A. FINKBEINER, New York, New York, U.S.A.
PIERRE HAOUR, Lyon, France

24. The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Breast Carcinoma after Adrenalectomy With or Without Additional Ovariectomy (Those Treated by Sex Steroids and Those Without Additional Sex Steroids Administration Considered Separately)

JOHN A. FINKBEINER, New York, New York, U.S.A.
PIERRE HAOUR, Lyon, France

25. The Cell Type (Normal Squamous Cells Only) in Vaginal Smears of Patients with Breast Carcinoma After Hypophysectomy With or Without Additional Adrenalectomy and/or Ovariectomy (Those Treated by Sex Steroids and Those Without Additional Sex Steroids Administration Considered Separately)

JOHN A. FINKBEINER, New York, New York, U.S.A.

26. Ovarian Function Following Pelvic Irradiation As Assessed by Vaginal Smears

ARTURO ANGEL ARRIGHI, Buenos Aires, Argentina
JOHN A. FINKBEINER, New York, New York, U.S.A.
J. PAUL PUNDEL, Luxembourg, Luxembourg

27. The Vaginal Smear During Menopause

HANNAH PETERS, Bombay, India

Symposium B

ADVANTAGES AND DISADVANTAGES OF VARIOUS TECHNIQUES OF OBTAINING MATERIAL FOR ROUTINE CYTOLOGICAL EXAMINATIONS

1. Review of Techniques of Vaginal Smears

ABRAHAM E. RAKOFF, Philadelphia, Pennsylvania, U. S. A.
HOWARD L. RICHARDSON, Seattle, Washington, U. S. A.
J. PAUL PUNDEL, Luxembourg, Luxembourg

Disc.: Marcel Gaudefroy, Lille, France
Emmerich von Haam, Columbus, Ohio, U. S. A.
Pierre Haour, Lyon, France
Guillermo Terzano, Buenos Aires, Argentina

2. Review of Techniques of Cervical Smears

HERBERT E. NIEBURGS, New York, New York, U. S. A.

Disc.: Arturo Angel Arrighi, Buenos Aires, Argentina
Emmerich von Haam, Columbus, Ohio, U. S. A.
Abraham E. Rakoff, Philadelphia, Pennsylvania, U. S. A.
Howard L. Richardson, Seattle, Washington, U. S. A.
Horst Smolka, Kiel, Germany

3. Review of Techniques of Endocervical Smears

HERBERT E. NIEBURGS, New York, New York, U. S. A.
HORST SMOLKA, Kiel, Germany

Disc.: Emmerich von Haam, Columbus, Ohio, U. S. A.
Abraham E. Rakoff, Philadelphia, Pennsylvania, U. S. A.
Howard L. Richardson, Seattle, Washington, U. S. A.

4. Review of Techniques of Intrauterine Smears

HERBERT E. NIEBURGS, New York, New York, U. S. A.
HOWARD L. RICHARDSON, Seattle, Washington, U. S. A.

Disc.: Pierre Haour, Lyon, France
Hannah Peters, Bombay, India
Abraham E. Rakoff, Philadelphia, Pennsylvania, U. S. A.

5. Material Obtained by Pipette from Vaginal Pool Only, With Visualization of Cervix

EMMERICH von HAAM, Columbus, Ohio, U. S. A.

Disc.: Marcel Gaudefroy, Lille, France
J. Paul Pundel, Luxembourg, Luxembourg

6. Material Obtained by Pipette from Vaginal Pool After Insertion of Speculum

J. PAUL PUNDEL, Luxembourg, Luxembourg

Disc.: Marcel Gaudefroy, Lille, France

7. Material Obtained by Vaginal Tampon

HERBERT E. NIEBURGS, New York, New York, U. S. A.

8. Material Obtained by Cervical Scraping

P. PAUL PUNDEL, Luxembourg, Luxembourg
HOWARD L. RICHARDSON, Seattle, Washington, U. S. A.

Disc.: Pierre Haour, Lyon, France
Warren C. Hunter, Portland, Oregon, U. S. A.

9. Material Obtained by Two Techniques: (a) Vaginal Smears
(b) Cervical Smears

EMMERICH von HAAM, Columbus, Ohio, U. S. A.
Y. S. SONG, Providence, Rhode Island, U. S. A.
STANLEY WAY, Newcastle, Upon Tyne, England, U. K.

Disc.: Pierre Haour, Lyon, France
Hannah Peters, Bombay, India
Wolfgang Walz, Heidenheim, Brenz, Germany

10. Material Obtained by Two Techniques: (a) Cervical Smears
(b) Endocervical Smears

EDMUND SCHÜLLER, Vienna, Austria

Disc.: Howard L. Richardson, Seattle, Washington, U.S.A.

11. Material Obtained by Three Techniques: (a) Vaginal Smears
(b) Cervical Smears (c) Endocervical Smears

JEAN de BRUX, Paris France
EMMERICH von HAAM, Columbus, Ohio, U.S.A.

Disc.: Pierre Haour, Lyon, France
Constantin Herovici, Villejuif, Seine, France
J. Paul Pundel, Luxembourg, Luxembourg
Abraham E. Rakoff, Philadelphia, Pennsylvania, U.S.A.

12. Material Obtained by Sponge-Biopsy

PETER STOLL, Heidelberg, Germany

13. If One Prepares More Than One Smear Per Patient, Could One Prepare These Smears on One Glass Slide or Does One Have to Put Them on Separate Glass Slides?

Y. S. SONG, Providence, Rhode Island, U.S.A.
GEORGE L. WIED and GUNTER F. BAHR, Chicago, Illinois, U.S.A.

Disc.: Marcel Gaudefroy, Lille, France
Emmerich von Haam, Columbus, Ohio, U.S.A.
Wolfgang Walz, Heidenheim a.d., Brenz, Germany

14. Material Obtained by Use of the Blade of a Bivalve Speculum and Smearing with Gloved Finger

WARREN C. HUNTER, Portland, Oregon, U.S.A.

Disc.: Howard L. Richardson, Seattle, Washington, U.S.A.
Horst Smolka, Kiel, Germany

15. In a Case of A Suspicious Ectocervix, Should A Biopsy Be Done Without Cytology?

Y. S. SONG, Providence, Rhode Island, U.S.A.
PETER STOLL, Heidelberg, Germany

Disc.: Marcel Gaudefroy, Lille, France
J. Paul Pundel, Luxembourg, Luxembourg

VOLUME IV 1960 NUMBER 1

The Symposium by Correspondence of this edition will be devoted to the discussion of

CARCINOMA IN SITU AND SO-CALLED PRECANCEROUS LESIONS

Deadlines for Contributors to the Symposia of this Edition:

FOR BEING LISTED AS SPEAKER OR DISCUSSANT:

In order to be listed as Main Speaker or Discussant on any of the topics listed in the following program, participants should inform the Editorial Office as soon as possible; however, NOT LATER THAN: July 1, 1959, about their intention to contribute.

FOR RECEIPT OF MAIN PAPERS:

Main papers in the English language must REACH ACTA CYTOLOGICA, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A., NOT LATER THAN: September 1, 1959.

Main papers in French, German or Spanish must REACH ACTA CYTOLOGICA NOT LATER THAN: July 20, 1959, to permit translation and subsequent approval by author.

FOR RECEIPT OF DISCUSSIONS:

THE DISCUSSANTS WILL RECEIVE THE PAPERS OF THE MAIN SPEAKERS FOR COMMENTS AS SOON AS THEY ARE AVAILABLE.

Discussions in the English language must REACH ACTA CYTOLOGICA, 5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A., NOT LATER THAN: November 15, 1959.

Discussions in French, German or Spanish must REACH ACTA CYTOLOGICA NOT LATER THAN: October 15, 1959, to permit translation and subsequent approval by author.

FOR RECEIPT OF CLOSING REMARKS BY MAIN SPEAKERS:

THE MAIN SPEAKERS WILL RECEIVE THE DISCUSSIONS FOR CLOSING REMARKS AS SOON AS THEY ARE AVAILABLE.

Closing remarks in the English language must REACH ACTA CYTOLOGICA NOT LATER THAN: February 1, 1960. Closing remarks in French, German or Spanish must REACH the Editorial Office NOT LATER THAN: January 1, 1960.

PRELIMINARY LIST OF TOPICS OF SYMPOSIA OF VOLUME IV, 1960, NUMBER 1

CARCINOMA IN SITU AND SO-CALLED PRECANCEROUS LESIONS

Introduction

SPECIAL PANEL DISCUSSION ON HISTOLOGICAL TERMINOLOGY (a specially invited panel will discuss the definitions of the following conditions):

Normal Epithelium
Ectopy and Ectropion
Epidermization and Atypical Epidermization
Leukoplakia and Parakeratosis
Reserve Cell Hyperplasia
Basal Call Hyperplasia
Abnormal Epithelium (School of Zürich)
Undifferentiated Regenerative Epithelium
Dysplasia
Carcinoma in Situ
Early Invasive Carcinoma (Microcarcinoma)
Invasive Carcinoma

The discussion of the above definitions will be based on photomicrographs and histomorphological descriptions. The definitions are not necessarily intended to become international standard terminology, but are merely intended to serve as introduction to the contributions to the following symposium.

Individual Topics

Symposium A

PROBABLE OR POSSIBLE PRE-MALIGNANT CERVICAL LESIONS

Cervicitis and Endocervicitis

1. Histomorphology of Cervicitis and Endocervicitis
2. Histochemistry of Cervicitis and Endocervicitis
3. Exfoliative Cytology of Cervicitis and Endocervicitis
4. Cytochemistry of Cervicitis and Endocervicitis
5. Colposcopy of Cervicitis and Endocervicitis
6. Colpomicroscopy of Cervicitis and Endocervicitis
7. Clinical Viewpoint: Management of Cervicitis and Endocervicitis
8. Interrelationship: Cervicitis-Endocervicitis and Cervical Carcinoma

Ectopy, Ectropion and Epidermization

9. Histomorphology of Ectopy, Ectropion and Epidermization
10. Histochemistry of Ectopy, Ectropion and Epidermization
11. Exfoliative Cytology of Ectopy, Ectropion and Epidermization
12. Cytochemistry of Ectopy, Ectropion and Epidermization
13. Colposcopy of Ectopy, Ectropion and Epidermization
14. Colpomicroscopy of Ectopy, Ectropion and Epidermization
15. Clinical Viewpoint: Management of Ectopy, Ectropion and Epidermization
16. Interrelationship: Ectopy-Ectropion-Epidermization and Cervical Carcinoma

Leukoplakia

17. Histomorphology of Leukoplakia
18. Histochemistry of Leukoplakia
19. Exfoliative Cytology of Leukoplakia
20. Cytochemistry of Leukoplakia
21. Special Microscopic Studies on Leukoplakia (Interference-, UV-, Electron-, Phase-, or Fluorescence-Microscopy)
22. Animal Experiments
23. Colposcopy of Leukoplakia
24. Colpomicroscopy of Leukoplakia
25. Clinical Viewpoint: Management of Leukoplakia
26. Interrelationship: Leukoplakia and Cervical Carcinoma

Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

27. Histomorphology of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
28. Histochemistry of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
29. Exfoliative Cytology of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
30. Cytochemistry of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
31. Special Microscopic Studies on Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia (Interference-, Electron-, Phase-, UV-, or Fluorescence-Microscopy)
32. Animal Experiments
33. Colposcopy of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

34. Colpomicroscopy of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
35. Clinical Viewpoint: Management of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia
36. Interrelationship: Reserve Cell Hyperplasia-Basal Cell Hyperplasia-Dysplasia and Cervical Carcinoma

Symposium B

PROBABLE OR POSSIBLE MALIGNANT CERVICAL LESIONS: CARCINOMA IN SITU

Histology of Carcinoma in Situ

1. Histomorphology of Carcinoma in Situ
2. What Constitutes a Definite Diagnosis of Carcinoma in Situ with Special Reference to Amount of Histological Sections Required ("Serial Section") and Type of Material?
3. What is Not Carcinoma in Situ?
4. What Percentage of Cervices Show Early Invasion in Serial Histological Sections in Uteri Which Were Removed Under the Biopsy Diagnosis of "Carcinoma in Situ"?
5. Nomenclature of the Atypical Epithelium
6. The Atypical Non-Invasive Zones Around Invasive Carcinoma (Cervical)
7. Histochemistry of Carcinoma in Situ
8. Special Microscopic Studies on Histological Sections of Carcinoma in Situ (Interference-, Electron-, UV-, Fluorescence-, or Phasemicroscopy)

Inspection Techniques on Carcinoma in Situ

9. Colposcopy of Carcinoma in Situ
10. Colpomicroscopy of Carcinoma in Situ
11. Schiller Test on Carcinoma in Situ

Exfoliative and Experimental Cytology of Carcinoma in Situ

12. Cytomorphology of Carcinoma in Situ
13. Cytochemistry of Carcinoma in Situ
14. Phasemicroscopy on Exfoliated Cells of Carcinoma in Situ
15. Ultraviolet Microscopy on Exfoliated Cells of Carcinoma in Situ
16. Fluorescence Microscopy on Exfoliated Cells of Carcinoma in Situ
17. Electron Microscopy on Exfoliated Cells of Carcinoma in Situ
18. Interference Microscopy on Exfoliated Cells of Carcinoma in Situ
19. Can One Differentiate Carcinoma in Situ from Invasive Carcinoma by Means of Exfoliative Cytology?
20. Carcinoma in Situ of the Uterine Cervix as the Primary Cytological Diagnosis
21. Exfoliative Cytology Following Irradiation of Carcinoma in Situ
22. Experimental Carcinoma in Situ (Animal Experiments)
23. Tissue Culture of Cells from Carcinoma in Situ

Clinical Viewpoint on Carcinoma in Situ

24. Advantages and Disadvantages of Conservative Management of Carcinoma in Situ (with special reference to possible recurrence, fatal outcome and postoperative pregnancy)
25. Advantages and Disadvantages of Radical Surgical Management of Carcinoma in Situ
26. Advantages and Disadvantages of Irradiative Management of Carcinoma in Situ
27. Management of Recurrent Carcinoma in Situ after Cone Biopsy

28. Advantages and Disadvantages for the Clinical Management of Cytologic Prediction of the Extent and Nature of a Cervical Lesion (Invasive or Non-Invasive) if this Prediction were Correct in at Least 80% of the Cases

29. May one Treat the "Early Invasive Carcinoma (Microcarcinoma)" Less Radically that the More Extensive Invasive Carcinoma?

Morphogenesis and Incidence of Carcinoma in Situ

30. Incidence of Carcinoma in Situ with Special Reference to Age, Pregnancy, Parity, Race and Hormonal Condition

31. Probable Percentage of Invasive Carcinoma Passing Through the Stage of Carcinoma in Situ

32. Latent Period of Carcinoma in Situ

33. Probable Percentage of Regression of Carcinoma in Situ

34. Do Surgical Procedures on Cervices with Carcinoma in Situ Increase the Incidence of Post-operative Invasions?

35. Cervical Carcinoma in Situ as Compared with Carcinoma in Situ in Other Sites

INDIVIDUALS WHO WISH TO PARTICIPATE IN THE SYMPOSIA BY CORRESPONDENCE AND WHO HAVE NOT YET RECEIVED AN INVITATION TO PARTICIPATE ARE REQUESTED TO WRITE TO THE EDITORIAL OFFICE (5841 SOUTH MARYLAND AVENUE, CHICAGO 37, ILLINOIS, U. S. A.) INDICATING THE SUBJECT TO WHICH THEY WISH TO CONTRIBUTE

SYMPOSIA UNDER CONSIDERATION

The following symposia have been suggested for consideration and are not listed in the order of preference or chronology. The readers are invited to inform the Editorial Office in which of the symposia they would be most interested, so that an order of preference may be tentatively arranged.

1. Symposium on Tadpole-Shaped Squamoid Cells.
2. Symposium on Organization of Laboratory of Exfoliative Cytology.
3. Symposium on Cytological Studies in Amenorrhea.
4. Symposium on Cytology of Ascitic Fluid.
5. Symposium on Cytology of Malignant Tumors of Ovary and Tubes.
6. Symposium on Extra-Genital Cytology of Metastatic Gynecological Lesions.
7. Symposium on Phasemicroscopy and Other Special Microscopic Techniques.
8. Symposium on Training of Exfoliative Cytologists.
9. Symposium on Cytological Changes due to Microbiological Factors.
10. Symposium on the Comparative Diagnostic Accuracy, Efficiency and Specificity of Techniques for Detection of Carcinoma.
11. Symposium on Histiocytes.
12. Symposium on Cytological Microphotography.
13. Symposium on Cytological Terminology for Hormonal Evaluation.
14. Symposium on Quantitative Cytochemistry of Exfoliated Cells.
15. Symposium on Sex Chromatin.
16. Symposium on Vaginal Cytology During Childhood.
17. Symposium on Lung Cytology.
18. Symposium on Cytology of Exudates.
19. Symposium on Gastrointestinal Cytology.
20. Symposium on Endocervical Adenocarcinoma.
21. Symposium on Training of Cytotechnicians.
22. Symposium on Effect of Progestational Agents on the Vaginal Epithelium.

THIS AND THAT

This portion of ACTA CYTOLOGICA is devoted to miscellaneous information.

INFORMATIONS DIVERSES

Cette rubrique des ACTA CYTOLOGICA est destinée à des informations diverses.

PERSÖNLICHE INFORMATIONEN

Dieser Teil der ACTA CYTOLOGICA befasst sich mit verschiedenen persönlichen Nachrichten.

ESTO Y AQUELLO

Esta parte de ACTA CYTOLOGICA está dedicada a información diversa.

INES L. P. de ALLENDE OF CORDOBA, ARGENTINA will visit several European countries in the spring and summer of 1959. Dr. de Allende will attend the meeting of the Third World Congress on Fertility and Sterility in Amsterdam in June of 1959. Dr. de Allende spoke on Diagnosis of Ovulatory Cycle by Means of the Colpocytological Method at the meeting of the Sociedad De Obstetricia y Ginecologica de Cordoba, Argentina in September, 1958.

THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS and the COLLEGE OF AMERICAN PATHOLOGISTS had their meeting in Chicago, November 3 to 7. Workshops on exfoliative cytology were sponsored by the Council on Cytology of the ASCP of which Dr. James W. Reagan of Cleveland, Ohio is currently chairman. Drs. Erickson, Simon and Woolner gave workshops and a seminar was conducted on Pulmonary Cytology. Dr. Lewis Woolner of Rochester, Minnesota gave this seminar and the participants were Dr. Koss, Dr. von Haam and Dr. Hazard.

GUNTER F. BAHR OF STOCKHOLM, SWEDEN visited the United States of America during the year 1958. Among other places, he has been with Drs. Ruth Graham, Koss, Reagan and Wied, and he attended the meeting of the Inter-Society Cytology Council in New York from November 13 through 15, prior to his return to Sweden.

HANNS-WERNER BOSCHANN OF WEST-BERLIN, GERMANY has been elected Chief of Service of the Department of Obstetrics and Gynecology, Rudolf-Virchow-Hospital, West-Berlin. Dr. Boschann participated in the meeting of the Inter-Society Cytology Council in New York from November 13 through 15, and gave a lecture on histochemistry of early carcinoma at the University of Chicago on November 21.

JOSÉ BOTELLA-LLUSIA OF MADRID, SPAIN gave guest lectures in Brazil at the Universities of Salvador, Rio de Janeiro and Sao Paulo during August of 1958. Dr. Botella gave the Presidential Address at the meeting of the Spanish Gynecological Society, October, 1958, on "New Gestagens." Professor Botella was elected Chevalier de la Legion d' Honneur in Paris, May, 1958.

JEAN de BRUX OF PARIS, FRANCE will conduct a course on exfoliative cytology in the beginning of next year. The course will be associated with a training course for cytotechnicians.

JORGE R. de C. OF LIMA, PERU has been elected Vice President of the Peruvian Society of Pathology. Dr. Campos attended the II Latin American Congress of Pathology in Sao Paulo, Brazil in September, 1958.

TORBJOERN O. CASPERSSON OF STOCKHOLM, SWEDEN participated in the meeting of the Inter-Society Cytology Council in New York from November 13 through 15, 1958. Dr. Caspersson became a member of an informal group which will plan an international cytology congress.

LOWELL T. COGGESHALL OF CHICAGO, ILLINOIS, U. S. A. has been appointed by the President of the United States of America as the Alternate United States Representative of the Executive Board of World Health Organization, November 7, 1958.

M. EDWARD DAVIS OF CHICAGO, ILLINOIS, U. S. A. has been elected President of the American Association of Maternal and Infant Health.

EMMERSON DAY OF NEW YORK, NEW YORK, U. S. A., who is the immediate Past President of the Inter-Society Cytology Council, became the chairman of a group which will plan an international cytology congress. The other members of the group are: Dr. Torbjoern O. Caspersson of Stockholm, Sweden, Dr. Julieta C. de Laguna of Mexico, D. F., Mexico, Dr. George N. Papanicolaou of New York, New York, Dr. J. Paul Pundel of Luxembourg, Luxembourg and Dr. George L. Wied, of Chicago, Illinois, U. S. A.

JACQUES FERIN OF LOUVAIN, BELGIUM spoke in Paris, (Hopital Broca) on the effects of the new progestational compounds on the vaginal smears, and in Kiel (Deutsche Gesellschaft für Endokrinologie) on "Vergleichende Wirsamkeit der neuen Gestagenen bei der Ovariektomierten Fray."

CLARICE do AMARAL FERREIRA OF RIO DE JANEIRO , BRAZIL is in charge of the organization of 1st Symposium on Cytology of the Sociedade Brasileira de Citologia.

JOHN A. FINKBEINER OF NEW YORK, NEW YORK, U. S. A. presented a paper on cytology in breast cancer at the meeting of the Inter-Society Cytology Council in New York from November 13 through 15.

ALVAN G. FORAKER OF JACKSONVILLE, FLORIDA, U. S. A. attended the International Cancer Congress in London. Dr. Foraker lectured on nuclear mass in cervical carcinoma to the Gynecology Clinic at the University of Paris on June 30 and to the Societa Lombarda de Ostetricaia e Ginecologia in Milan, Italy on August 1. A conference was held on "Research in a Community Hospital" at the Royal Infirmary, Department of Pathology, in Glasgow on July 16, 1958.

MARCEL GAUDEFROY OF LILLE, FRANCE is a Founding Member of the European Society of Clinical Cytology.

HEINZ GRUNZE OF WEST-BERLIN, GERMANY who is currently a Fulbright Research Fellow with Dr. Wied in Chicago, participated in a meeting of the Inter-Society Cytology Council in New York, November 13-15.

EMMERICH von HAAM OF COLUMBUS, OHIO, U. S. A. was visiting professor at the University of Washington in Seattle, where he participated on a symposium on clinical cytology during August, 1958. Dr. von Haam addressed the Williamson, W. Va. Medical Society on "Cytology - A New Diagnostic Tool" on September 17 and conducted a discussion on cytology on board the S. S. Stockholm as a faculty member of the Medical Seminar Cruise, November 11-15, 1958.

HANS IGEL OF BERLIN, GERMANY will participate in the International Congress for Sterility and Fertility in Amsterdam in 1959.

LEOPOLD G. KOSS OF NEW YORK, NEW YORK, U. S. A. participated in the Congress of Pathology in Chicago in November and was the program chairman of the meeting of the Inter-Society Cytology in New York on November 13-15. Dr. Koss was elected Assistant Secretary of the Inter-Society Cytology Council.

JULIETA CALDERON de LAGUNA OF MEXICO, D. F., MEXICO participated in the 28th National Medical Congress in San Jose in Costa Rica, and in the meeting of the Inter-Society Cytology Council in New York and the 13th Asamlea Nacional de Cirujanos in Mexico - all three meetings in November, 1958. Dr. de Laguna is President of the Mexican Society of Cytology and President of the Sociedad Medica del Hospital de la Mujer, Dr. J. Aleman Perez.

WARREN R. LANG OF PHILADELPHIA, PENNSYLVANIA, U. S. A. participated in a two-week course at Massachusetts Institute of Technology on "Research Methods in Biology and Medicine," and spoke on Premenarchal Vaginitis, District III ACOG, October 3, 1958, at Princeton, New Jersey. Dr. Lang is currently engaged in the preparation of a proposed symposium on "The Vagina" for the New York Academy of Sciences in April, 1959.

PAUL LOPES-CARDOZO OF LEYDEN, HOLLAND conducts a 3 day course on clinical cytology every year with demonstration of slides and lectures.

JOHN R. MCDONALD OF DETROIT, MICHIGAN, U. S. A. has been elected President of the Inter-Society Cytology Council. The next meeting of the Inter-Society Cytology Council will take place in Detroit, Michigan. The address of Dr. McDonald is: Harper Hospital, 3825 Brush Street, Detroit, Michigan, U. S. A.

ELIZABETH A. MCGREW OF CHICAGO, ILLINOIS, U. S. A. and her research group has been awarded the Hekton Gold Medal for original research at the convention of the American Medical Association in San Francisco in June, 1958. Dr. McGrew and her group were also the recipients of the First Award for Outstanding Achievement in Cytology for the work on tumor cells in the circulating blood. She was elected Vice-President for Cytology of the Inter-Society Cytology Council at the meeting of the Council in New York in November, 1958.

JOSÉ MARIA E. MEZZADRA, Professor of Obstetrics and Gynecology, Buenos Aires, Argentina, died in a plane crash in Argentina in January, 1959.

ROBERT C. MELLORS OF NEW YORK, NEW YORK, U.S.A. belongs to Research Advisory Committee on Lung Cancer, American Cancer Society, New York, New York.

LUIS MONTALVO-RUIZ OF MADRID, SPAIN conducted a course on cytology in the University of Madrid in October. Dr. Montalvo became gynecologist in charge of one of the services in the Municipal Maternity Hospital of Madrid, of which Professor Botella-Llusia is Director. As a consultant of the Health Service (Seguro de Enfermedad), Dr. Montalvo started a study on cytology during pregnancy and screens an average of 3000 pregnant women per annum.

VIOLETTE M. NUOVO OF PARIS, FRANCE participated in the meeting of the Inter-Society Cytology Council in New York on November 13-15 and presented a paper on pregnancy and cervical carcinoma.

ABRAHAM E. RAKOFF OF PHILADELPHIA, PENNSYLVANIA, U.S.A. has been elected President-Elect of the Inter-Society Cytology Council.

JAMES W. REAGAN OF CLEVELAND, OHIO, U.S.A. was elected to the Executive Committee of the Inter-Society Cytology Council on November 14, 1958.

EDMUND SCHÜLLER OF VIENNA, AUSTRIA became Professorial Lecturer (Dozent) in Obstetrics and Gynecology in February, 1958.

HORST SMOLKA OF KIEL, GERMANY participated in the International Meeting organized by the Société Royale Blege de Gynecologie et d'Obstetrique on the occasion of the World's Exhibition in Brussels on July 17, 1958. He presented a paper on "The Prognostic Judgment of Genital Cancer by Exfoliative Cytology." Dr. Smolka took part in the Deutsche Therapiewoche in Karlsruhe, Germany, August 31 through September 6, 1958, and reported on "Cytology as an Aid to the Treatment of Leukorrhea."

YOSEUP SONG OF PROVIDENCE, RHODE ISLAND, U.S.A. served as moderator at a Cytology Workshop for the Massachusetts Society of Pathologists at Harvard Medical School and participated in the Annual Meeting of the Inter-Society Cytology Council in November, 1958.

PETER STOLL OF HEIDELBERG, GERMANY became (apl.) Professor of Obstetrics and Gynecology. He participated in the German Gynecology Congress in Frankfurt in September, 1958 and presented a paper on phase microscopy at the meeting of the Inter-Society Cytology Council in New York in November, 1958.

GUILLERMO TERZANO OF BUENOS AIRES, ARGENTINA participated in the Round Table Conference on Cancer Cytology in Buenos Aires and in the meeting of the Inter-Society Cytology Council in New York in November, 1958.

ERICA G. WACHTEL OF LONDON, ENGLAND visited Lima, Peru and participated in the meeting of the Inter-Society Cytology Council in New York in November, 1958.

ROBERT WENNER OF BASEL, SWITZERLAND visited the United States of America, especially the University Hospitals of Boston, New York, Washington, Baltimore, Chicago and Salt Lake City, during the summer of 1958.

GEORGE L. WIED OF CHICAGO, ILLINOIS, U.S.A. participated in the meeting of the Detroit Cancer Institute in October 28, 1958 and the meeting of the Inter-Society Cytology Council in New York, November 13-15, 1958. He has been elected a member of the Executive Committee of the Inter-Society Cytology Council. Dr. Wied has been invited to give a cytology course in Melbourne, Australia in the spring of 1959.

PAUL A. YOUNGE OF BROOKLINE, MASSACHUSETTS, U.S.A. was elected Secretary-Treasurer of the Inter-Society Cytology Council. The address of Dr. Younge is: 1101 Beacon Street, Brooklyn 46, Massachusetts, U.S.A.

HANS KLAUS ZINSER OF COLOGNE, GERMANY will present a paper on cancer cytology at the German Cancer Congress in Berlin in the spring of 1959.

Upon request,
the Editorial Office
will furnish the mailing addresses
of the mentioned individuals.
Pertinent contributions
to the above section
THIS AND THAT
are invited.

WANTED OR AVAILABLE

It is the purpose of this column to promote international exchange of cytologists and cytotechnicians, to inform them of open permanent positions, and to inform employers of available cytology personnel. Persons interested in obtaining permanent positions as cytologists or cytotechnicians or in obtaining temporary fellowships in cytology (teaching, exchange, or training fellowships), and individuals or institutions offering such positions or openings are invited to write giving full information to: ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A. Information supplied will be held strictly confidential.

While information received is subject to editing so that it conforms to the style of ACTA CYTOLOGICA, ACTA CYTOLOGICA cannot and do not assume responsibility for statements made by contributors.

OFFRES ET DEMANDES

Cette rubrique est destinée à favoriser l'échange international de cytologistes et de techniciens en cytologie. Elle renseignera sur les places permanentes vacantes et informera également sur le personnel cytologique disponible. Les personnes désirant obtenir une place permanente de cytologiste ou cytotechnicien, ou faire un stage temporaire en cytologie (enseignement, échange, training), et les instituts ou personnes offrant de telles places sont invités à écrire aux ACTA CYTOLOGICA (5841, Maryland Avenue, Chicago 37, Illinois, U.S.A.) en donnant tous les détails. Les informations reçues auront un caractère strictement confidentiel.

Les annonces reçues devront être, pour la publication, rédigées dans le style des ACTA CYTOLOGICA, mais les ACTA CYTOLOGICA ne peuvent accepter aucune responsabilité pour l'exactitude des renseignements fournis par les annonceurs.

STELLENANGEBOTE UND STELLENGESUCHE

Mit dieser Rubrik soll internationaler Stellenaustausch und Stellenvermittlung für Zytologen und zytologisch-technische Assistenten angebahnt werden, indem über offene Stellungen und über verfügbares Personal berichtet wird. Zytologen und zytologisch-technische Assistenten und Assistentinnen, die an vorübergehenden (Lehrstellen, Austauschstellen, Lernstellen) oder dauernden Anstellungen interessiert sind, und Personen oder Institutionen, die derartige Stellunge zu vergeben haben, sind gebeten an ACTA CYTOLOGICA (5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A.) zu schreiben und möglichst genaue Einzelheiten anzugeben. Die erhaltenen Auskünfte und Einzelheiten werden streng vertraulich behandelt.

ACTA CYTOLOGICA kann keine Verantwortung für Angaben übernehmen, die von Beitragenden zu dieser Rubrik gemacht werden.

SOLICITUDES Y OFERTAS

El propósito de esta sección es promover el intercambio internacional de citólogos y técnicos en Citología, informar de vacantes en puestos permanentes, y de personal citológico disponible. Las personas que estén interesadas en obtener becas temporales, o puestos permanentes como citólogos o técnicos en Citología (Enseñanza, Intercambio, Becas de aprendizaje), y, asimismo, las personas o instituciones que puedan ofrecer tales puestos, deben escribir a ACTA CYTOLOGICA (5841 Maryland Avenue, Chicago 37, Illinois, USA) aportando información completa. Esta información será estrictamente confidencial.

Cuando las informaciones recibidas sean para su publicación en ACTA CYTOLOGICA, la Revista, no puede asumir, ni asume, la responsabilidad de los informes o afirmaciones hechas por los contribuyentes.

CYTOLOGISTS AND CYTOTECHNICIANS WANTED

TRAINEES IN EXFOLIATIVE CYTOLOGY WANTED in Laboratory of Exfoliative Cytology in a Medical School in the United States of America. The prospective applicants must be either citizens of the United States of America, or have taken out citizenship papers (immigrant); must be high school graduate or have equivalent credits with some training in biological sciences; no physical or mental disabilities that would interfere with training or restrict services as a cytology technician after training. Trainees will be awarded a stipend for a period of six months at the rate of $225 per month.

Write to: ACTA CYTOLOGICA 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A. and refer to: U.S. Public Health Service Traineeship.

CYTOTECHNICIANS WANTED for the Laboratory of Exfoliative Cytology of the University of Chicago Clinics. The prospective applicants be high school graduates with 18 semester hours of courses in Biological sciences who want to make cytology his or her career or/and who has had previous training in exfoliative cytology. The salary is adjusted according to the regulations of the University of Chicago Personnel Office.

Write to: George L. Wied, M.D., Chicago Lying-in Hospital, Chicago 37, Illinois, U.S.A.

CHIEF CYTOTECHNICIAN WANTED for the Cytology Laboratory of the Cancer Cytology Survey Program of Rhode Island. This senior cytology checker will be given a supervising duty as well as an opportunity to teach cytology students. The salary is $4862 per annum. The work week is 35 hours with two weeks and three days vacation per year.

Write for details to: Dr. Y. S. Song, Director in Charge (Pathologist) for Cancer Cytology Survey of Rhode Island, 593 Eddy Street, Providence 2, Rhode Island.

MEDICAL RESEARCH TECHNOLOGIST WANTED trained or willing to be trained as a cytotechnician for work in the Department of Obstetrics and Gynecology at L.S.U. School of Medicine; Louisiana State Civil Service Employee; starting salary $3500.

QUALIFIED CYTOTECHNICIAN WANTED full-time for Department of Pathology of Medical College of Georgia, Augusta. Opportunities for research and teaching in medical school environment. New Laboratories, well-equiped and air-conditioned. Attractive salary.

Apply: Dr. L. D. Stoddard, Professor of Pathology, Medical College of Georgia, Augusta, Georgia.

RESEARCH FELLOW IN GYNECOLOGIC PATHOLOGY WANTED. The Baptist Memorial Hospital, Jacksonville, Florida, will offer a Research Fellowship in the general area of research in gynecologic pathology to begin in the summer of 1959. The hospital currently operates 272 beds. It was opened in September, 1955, in a new air-conditioned building directly on the St. Johns River. The laboratories are attractively located on the second floor with separate laboratories for Histology, Bacteriology, Hematology, Clinical Microscopy and Chemistry. A new research laboratory with animal facilities is located on the sixth floor. The hospital is approved for residency training in Pathologic Anatomy and Clinical Pathology. Requirements: Graduation from an approved medical school, one year of approved internship, and at least one year of approved residency training in pathology or in obstetrics-gynecology. Stipend: A stipend of $3800 per year with an additional $350 per year for one or two dependents. No maintenance is furnished. Uniforms are not furnished but are laundered without charge.

Applications and inquiries should be addressed to: Alvan G. Foraker, M.D., Pathologist, Baptist Memorial Hospital, 800 Miami Road, Jacksonville 7, Florida.

CYTOLOGISTS AND CYTOTECHNICIANS AVAILABLE

GYNECOLOGIST, Male, Age: 34, citizen of West Germany, single, graduate of West German Medical School (M.D. - Dr. Med.)

Medical Training:	German Board of Obstetrics and Gynecology (Facharzt). Currently with University Dept. OB. & Gyn.
Wanted:	Traineeship (Paid Fellowship) in Exfoliative Cytology for the period of one year.
Code No.:	JMD 2/1/58, in care of ACTA CYTOLOGICA, 5841 South Maryland, Chicago 37, Illinois, U.S.A.

CYTOLOGIST-GYNECOLOGIST, Female, Age: 34, citizen of West Germany, single, graduate of West German Medical School (M.D. - Dr. Med.)

Medical Training:	Eligible for German Board of Obstetrics and Gynecology (Facharzt fuer Frauenheilkunde). Completed additional internship and full residency training in Obstetrics and Gynecology in the United States of America.
Cytology Training:	(1) in West German Medical School, Department of Obstetrics and Gynecology and (2) one and a half years full time Cytology Fellowship in a training center in the United States (approved by the American Cancer Society).
Wanted:	Position as gynecologist in charge of Cytology Laboratory (with clincial work if desirable) in The United States of America, Canada or West-Berlin, Germany.
Code No.:	OMD 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.

CYTOLOGIST-PATHOLOGIST, Male, Age: 36, single, graduate from Italian University (M.D.), citizen of Italy.

Medical Training:	Pathologist with six years experience after completion of residency.
Cytology Training:	Self-trained.
Wanted:	Training Fellowship in Cytology or Research Associate in Cytology in Austria, Germany or Switzerland for 6 to 12 months.
Code No.:	LLM 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.

GYNECOLOGIST, Male, Age: 33, citizen of Denmark, married, graduate of Danish University (M.D.).

Medical Training:	Completed internship and residency in Obstetrics and Gynecology, and 3-1/4 years staff member in University Hospital.
Cytology Training:	None.
Wanted:	Training Fellowship in Cytology in Great Britain or Central Europe.
Code No.:	ANV 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.

CYTOTECHNICIAN, Female, Age: 25, single, citizen of the United States of America, University Graduate.

Cytology Experience:	Presently Chief-Cytotechnician in cytology Laboratory in a Medical School in the United States of America. This particular laboratory is a training laboratory approved by the American Cancer Society, co-author of several scientific publications on cytology.
Wanted:	Teaching Fellowship for 3-4 months to organize, set-up, or modernize cytology laboratories. Would consider India, Australia, New Zealand, Ireland or Africa. Will return to present position in United States upon completion of fellowship.
Code No.:	UG 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.

CYTOTECHNICIAN, Female, Age: 32, citizen of West Germany, single, registered medical technician.

Cytology Experience:	Chief-Cytotechnician 7 years in cytology laboratory of a University Department of Obstetrics and Gynecology in Germany. Experienced in cancer cytology, endocrinological cytology and hematology.
Wanted:	Exchange fellowship for a period of several months with a cytology center in the United States of America, Brazil or Argentina. Will return to present position upon completion of fellowship.
Code No.:	RU 1/1/57, in care of the ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A.